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ROLE OF CARBON-ONE AND CARBON-TWO COMPOUNDS IN THE  
BIOSYNTHESIS OF AMINO ACIDS IN HIGHER PLANT TISSUES

by

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FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled ROLE OF CARBON-ONE AND CARBON-TWO COMPOUNDS IN THE BIOSYNTHESIS OF AMINO ACIDS IN HIGHER PLANT TISSUES submitted by Suresh Kumar Sinha in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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## ABSTRACT

In the present investigations, the role of certain carbon-one and carbon-two compounds in amino acid biosynthesis, was assessed using a variety of higher plant tissues.

Plant tissues were shown to produce formate from glyoxylate and glycine and to utilize this formate in the biosynthesis of serine and methionine. Incorporation of formate- $C^{14}$  into these amino acids was enhanced by additions of homocysteine and glycine. The bulk of carbon- $^{14}$  present in the serine- $C^{14}$  obtained from formate- $C^{14}$  was recovered in the 3 position. The results are interpreted as indicating that formate can be utilized by these tissues for amino acid biosynthesis by pathways involving transmethylation reactions.

Studies on glycine- $C^{14}$  metabolism showed that this compound can be incorporated into all three positions of the serine molecule. In some tissues, glycine- $C^{14}$  was a better precursor for the 3 position of serine than was glyoxylate. The results, therefore, indicated the possibility of a direct cleavage of glycine into a carbon-one unit which can be utilized for synthesis of the 3 position of serine.

In glyoxylate- $C^{14}$  feeding experiments employing a variety of plant tissues, it has been shown that this compound is important in the synthesis of glycine and serine. In addition to this major pathway, glyoxylate was also reduced to glycollate and oxidized to oxalate in several of the tissues. In tissues known to be converting fat to carbohydrate via a





glyoxylate cycle, glyoxylate- $C^{14}$  apparently played only a minor role in sugar biosynthesis.

Extracts prepared from plant tissues, known to produce glycine from glyoxylate in vivo, were found to contain an active glyoxylate-transaminase system. The properties of this enzyme system have been studied.

Acetate-2- $C^{14}$  and carbon dioxide- $C^{14}$  also played an important role in amino acid biosynthesis in tissues known to contain the enzymes of the glyoxylate cycle. The possible pathways for the utilization of these compounds in amino acid biosynthesis have been discussed.



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## INTRODUCTION

Studies with animal tissues and microorganisms have revealed certain basic mechanisms for the biosynthesis of amino acids. For example, reactions involving amination, transamination, transmethylation, transthiolation and decarboxylation of amino acids, are of considerable importance in amino acid biosynthesis and interconversions (Fruton and Simmonds 1958, Bonner 1950, Conn and Stumpf 1963).

In plants, reductive amination of  $\alpha$ -ketoglutaric acid has been studied in detail and is apparently the main route for the entry of amino nitrogen into carbon skeletons (Yemm and Folks 1958, Virtanen 1961). Various transamination reactions involving glutamate, aspartate and alanine have also been studied and found to be of widespread occurrence (Yemm and Folks 1958, Meister 1955, Sakami and Harrington 1963). However, the present knowledge of the exact mechanism and occurrence of transmethylation reactions and various interconversions of amino acids such as glycine, serine and methionine is still far from complete.

From studies with animal tissues and microorganisms, it is apparent that carbon-one compounds (formate, formaldehyde and carbon dioxide) and carbon-two compounds (acetate, glyoxylate, glycine etc.) can play significant roles in the biosynthesis of certain amino acids (Sakami and Harrington 1963, Kornberg 1957). Recent studies by a number of workers for example, Rabson, Tolbert and Kearney (1962), Kearney and



Tolbert (1962), Wang and Waygood (1962) and Wang and Burris (1963) using plant tissues carrying out photosynthesis have suggested that formate, glyoxylate and glycine play important metabolic roles in the biosynthesis of amino acids. Furthermore, these compounds are now known to be produced in non-green plant tissues (Beevers 1961). However, studies on the role of these compounds in transmethylation and transamination have not been extensive.

The present studies were therefore conducted in an attempt to assess the importance of these carbon-1 and carbon-2 compounds in the biosynthesis of amino acids using a variety of plant tissues.





## REVIEW OF LITERATURE

In the present investigation, an effort has been made to assess the role of carbon-one and carbon-two compounds (formate, carbon dioxide, glyoxylate, acetate and glycine) in amino acid biosynthesis in higher plant tissues. Since most of the work in this field has been done with microorganisms and animal tissues, the literature cited is in part derived from these sources.

### The Role of Formate in Amino Acid Biosynthesis

Formate is known to play an important role in trans-methylation reactions in animal tissues. Studies with formate- $C^{14}$  supplied to rats have indicated that the 3 position of serine is produced from this compound in the presence of glycine (Alexander and Greenberg 1955, Kisluik and Sakami 1955). However, data reported by these workers and by Siegel and Lafaye (1950) have indicated that formaldehyde is a better precursor of the 3 position of serine than formate or methanol. It has therefore been suggested that in mammalian tissues, the one-carbon unit utilized for serine synthesis is reduced to the level of an active 'formyl' radical prior to condensation with glycine to yield serine.

In addition to this work on serine formation, Welch and Sakami (1950) have also demonstrated synthesis of the methyl carbons of methionine and choline when rats were injected with formate- $C^{14}$  or when liver slices were incubated with this radioactive compound together with homocysteine, dimethyl amino ethanol, folic acid and Vitamin  $B_{12}$ .



Incorporation of formate-C<sup>14</sup> into the carboxyl group of glyceric acid and into the 3 position of serine by barley leaves has been shown by Tolbert (1955). The pathway for incorporation of the label into the products appeared to utilize light. In darkened tissues, only relatively small amounts of formate entered the 3 position of serine. Recently McConnell and Bilinski (1959) have injected formate-C<sup>14</sup> into wheat plants and extracted the tissues after a 45 to 51-day period of growth. Formate-C<sup>14</sup> was incorporated into a variety of compounds including serine, methionine, glycine and histidine.

Studies by Doman and Romanova (1962) have shown that formic acid vapors are metabolized by bean and barley leaves in the light. They suggested that formate is oxidized to carbon dioxide and then is assimilated photosynthetically. In addition to this major pathway, small amounts of formate-C<sup>14</sup> were incorporated into serine.

Apart from these studies, metabolism of formate in vivo has been examined in other plant tissues, generally under conditions of illumination. Zbinovsky and Burris (1952) infiltrated several carbon-14 labelled organic acids into tobacco leaves. In the light, formate-C<sup>14</sup> was rapidly incorporated into citrate and malate. The intramolecular distribution of carbon-14 in malate so formed, indicated that formate was incorporated by a mechanism other than via carbon dioxide fixation (Burris 1953). It has also been reported by Krotkov, Vittorio and Reed (1954) that starch was labelled when formate-C<sup>14</sup> was supplied to illuminated tobacco leaves.

Therefore, various studies on formate metabolism in





plants indicate that this compound may be used in the biosynthesis of serine and also organic acids. Since, almost all of the studies have been conducted with tissues carrying on photosynthesis in light, it is difficult to conclude if formate is utilized as such or by photo-assimilation following oxidation to carbon dioxide. Clearly, studies with non-green tissues would eliminate the possibility of photo-assimilation and might be preferable for investigations on the role of formate in amino acid biosynthesis.

#### The Role of Glyoxylate in Amino Acid Biosynthesis

Glyoxylate is now known to play a significant role in intermediary metabolism (Beevers 1961). A glyoxylate transaminase system has been demonstrated in microorganisms such as *Pseudomonas* and *Neurospora* (Campbell 1956). In *Blastocladiella*, McCurdy and Cantino (1960) have also observed a glyoxylate-alanine transaminase system. The enzyme would apparently only transaminate the amino group of alanine. In agreement with work carried out on other transaminases, the glyoxylate-alanine transaminase was inhibited by hydroxylamine and had a pH optimum in the alkaline range. Frankel, Jilge and Eichhorn (1961) have also reported a glyoxylate transaminase system from *Aspergillus niger* which could utilize a number of amino acids as amino donors. For example, the enzyme could produce glycine from glyoxylate and the amino groups of alanine, glutamic acid,  $\gamma$ -amino butyric acid, glutamine and asparagine. Recently, Sastry and Ramakrishnan (1961) have described a metal dependent glutamic-glycine transaminase from green gram (*Phaseolus radiatus*). Thus, glyoxylate, by a transamination reaction, can



be converted into glycine by a number of bacterial and fungal species. The recent report of Sastry and Ramakrishnan, together with the data presented in this thesis, indicates that this enzyme system is widespread in higher plants also.

In experiments with silkworm tissues, Muramatsu and Shimura (1962) have studied the role of glyoxylate- $C^{14}$  in the biosynthesis of glycine and serine. These studies indicated a rapid incorporation of glyoxylate into the glycine and serine of the body fluid and the silk gland.

In tomato fruits, Doyle, Huff and Wang (1960) studied the role of glyoxylate in organic acid metabolism. Although only small amounts of glyoxylate- $C^{14}$  were incorporated into the organic acids, there was a considerable incorporation into the amino acid fraction. This fraction mainly consisted of glycine and serine.

In a recent paper, Kornberg and Morris (1963) have suggested a new pathway for the biosynthesis of amino acids from glyoxylate. Using Micrococcus denitrificans, they showed the synthesis of  $\beta$ -hydroxyaspartate from glyoxylate. They suggested that this resulted from a condensation of glyoxylate and glycine, leading to the production of  $\beta$ -hydroxyaspartate.

In addition to these studies, Tolbert, Clagett and Burris (1949) have shown the production of formate from glycollate. The enzyme catalyzed reaction involved the intermediary formation of glyoxylate. An enzyme system, catalyzing this oxidation, has been shown to occur in some plant tissues (Zelitch 1955).

◦ On the basis of studies conducted with photosynthesis-





ing tissues, microorganisms and animal tissues, it is therefore apparent that glyoxylate is utilized for the biosynthesis of certain amino acids. However, glyoxylate may also be involved in other important pathways (Beevers 1961, Millerd, Morton and Wells 1963). It is therefore of considerable interest to assess the role of this compound in the intermediary metabolism of non-green tissues such as germinating seeds where amino acid biosynthesis and interconversions accompany the germination process.

#### The Role of Carbon Dioxide in Amino Acid Biosynthesis

In green plants, carbon dioxide is utilized in the process of photosynthesis for the anabolism of important cellular constituents. It is now known that the first stable product of this photosynthetic carbon dioxide fixation is phosphoglyceric acid (Bassham and Calvin 1957, Bassham 1963). However, detailed studies on the kinetics of photosynthetic  $\text{CO}_2$  fixation have indicated that there is also an incorporation of label into amino acids. Vernon and Aronoff (1950), Towers and Mortimer (1956) have observed that in short term photosynthetic studies, alanine, serine and glycine were early products of  $\text{C}^{14}\text{O}_2$  fixation in soyabean leaves. After 15 seconds, the specific activities of these compounds were in the order alanine serine glycine. It has therefore been suggested that alanine is an earlier product than either glycine or serine (Vernon and Aronoff 1950). Since it is well established that glutamic acid-alanine transaminase systems are widespread in plant tissues (Loomis and Stumpf 1958), it is possible that phosphoglyceric acid is readily converted to pyruvate and eventually to alanine.



Labelling of glycine was explained on the basis that it might arise via glyoxylate produced from ribulose-5-phosphate as a result of the transketolase reaction (Weissbach and Horecker 1955).

Recently, Rabson, Tolbert and Kearney (1962) and Kearney and Tolbert (1962) have demonstrated the production of glycollic acid in isolated chloroplasts and have suggested that glycine, arising in photosynthesis, might be produced from glycollate via glyoxylate. Serine might then arise from glycine by condensation with an active 'formyl' radical produced from glycollate (Tolbert and Cohan 1953).

Dark fixation of carbon dioxide by various plant tissues has attracted much attention during the last decade (Ranson and Thomas 1960). In darkened sunflower seedlings, an exposure of 5 seconds to  $C^{14}O_2$  produced labelled alanine (Bradbeer 1958), while in germinating pea cotyledons, aspartic acid had the highest specific activity of the compounds produced in 5 minutes dark  $C^{14}O_2$  fixation (Cossins 1964). In longer periods of dark  $CO_2$  fixation, other amino acids and amides such as glutamate, asparagine and glutamine are produced. In excised soyabean leaves, carbon dioxide- $C^{14}$  fixation in the dark also leads to considerable synthesis of amino acids (Racusen and Aronoff 1954). The greatest amount of radioactivity derived from supplied  $C^{14}O_2$  was found in arginine. In addition, glutamate, aspartate, asparagine, serine, glycine and alanine were also labelled. On the basis of the distribution of carbon-14 in arginine, Racusen and Aronoff (1954) suggested that an ornithine cycle was operating in soyabean leaves.

It is therefore evident that assimilation of carbon





dioxide in light and dark can play a significant role in amino acid biosynthesis in plant tissues.

### The Role of Acetate in Amino Acid Biosynthesis

Acetate is extensively metabolized in living tissues following conversion to acetyl co-enzyme A. In actively growing plant tissues, acetate is therefore commonly metabolized by the reactions of the tricarboxylic acid cycle (Harley and Beevers 1963, Beevers 1961). As the operation of this cycle results in the production of keto-acids such as  $\alpha$ -ketoglutarate and oxaloacetate, the acetate carbon can be incorporated into glutamate and aspartate by transamination reactions. Bilinski and McConnell (1958) have demonstrated the formation of glutamate- $C^{14}$  from acetate- $C^{14}$  injected into wheat plants. Similarly, Canvin and Beevers (1961) have reported labelling of glutamate in castor bean endosperm tissues incubated with acetate. The operation of the tricarboxylic acid cycle together with carbon dioxide fixation leads to considerable synthesis of glutamate- $C^{14}$  from acetate- $C^{14}$  in germinating pea cotyledons (Cossins and Cameron 1964). Alanine, glutamine and asparagine are also important products of acetate metabolism in germinating sunflower cotyledons (Cossins and Sinha 1964).

Therefore, it is obvious that acetate may be used in the biosynthesis of amino acids in various plant tissues. However, if only the TCA cycle is operating for the metabolism of acetate, no net synthesis of aspartate or glutamate can occur. Clearly, if the TCA cycle is supplemented with dark  $CO_2$  fixation, a net synthesis of these amino acids could be





achieved. Similarly, in tissues where the glyoxylate cycle is operating, amino acid biosynthesis might be readily achieved by a drain on the cycle intermediates.

It is therefore of importance to assess the role of acetate in the amino acid biosynthesis using tissues known to contain the enzymes of the TCA cycle, glyoxylate cycle and enzymes for dark CO<sub>2</sub> fixation.

### The Role of Glycine in Plant Metabolism

Apart from playing an important role as a constituent of plant proteins, glycine is involved in several important reactions of intermediary metabolism. For example, glycine can enter into organic acids metabolism by transamination reactions which are known to occur in microorganisms (Campbell 1956, McCurdy and Cantino 1960). In Diplococcus glycinophilus, the glycine molecule is directly cleaved into carbon dioxide and a carbon-one unit without the intermediary formation of a carbon-two acid (Sagers and Gunsalus 1961).

Although considerable information has been obtained on the role of glycine in the biosynthesis of serine in animal tissues (Sakami 1955), glycine-serine interconversions have not been extensively studied in plant tissues. In animal tissues, it has been demonstrated that tetrahydrofolic acid is involved in the condensation of carbon-one unit with glycine (Sakami 1955).

In plants, McConnell and Bilinski (1959) have demonstrated the synthesis of serine from glycine-C<sup>14</sup> and formate-C<sup>14</sup>. They conclude from their data that the intact glycine molecule is used in the synthesis of serine. The reaction for the



interconversion of glycine and serine has been presumed to be freely reversible in wheat (Nath and McConnell 1960, McConnell and Finlayson 1961). Wang and Burris (1963) have also clearly demonstrated that serine was among the first compounds labelled in experiments when glycine-2-C<sup>14</sup> was fed to wheat leaves in light. However, feeding experiments with serine-1-C<sup>14</sup> did not produce any significant amount of glycine-1-C<sup>14</sup>. Therefore, they conclude that the enzyme catalyzed reactions for interconversion of glycine and serine are essentially irreversible in the tissues studied.

Recently, Wilkinson and Davies (1958, 1960) have extracted the enzyme hydroxymethyl tetrahydrofolic dehydrogenase from turnip which catalyzes the conversion of glycine to serine and is reversible. Similar results were obtained in experiments using extracts of immature cauliflower buds.

Investigations on glycine metabolism in higher plant materials have therefore been largely conducted with chlorophyll containing tissues, under conditions of illumination. As several investigations on the pathway of carbon in photosynthesis (Buchanan et al 1952, Towers and Mortimer 1956) have demonstrated that glycine, glycollate and serine are among the early products of carbon dioxide assimilation in the light, this might have important effects on the pathways for glycine metabolism in these tissues. Therefore, a study of glycine metabolism in non-photosynthetic tissue would be useful in regard to its possible transaminations and interconversions.



## MATERIALS AND METHODS

### Materials

In the present investigation, a variety of plant tissues have been used to assess their ability to metabolize various carbon-14 labelled compounds and to synthesize amino acids. The names of the plants and the tissues used in the present studies are given in Table 1.

### Labelled Compounds

Formate-C<sup>14</sup> and sodium carbonate-C<sup>14</sup> were purchased from Atomic Energy of Canada Ltd., Ottawa. Glyoxylate-1,2-C<sup>14</sup>, glycine-1,2-C<sup>14</sup>, glycine-2-C<sup>14</sup> and acetate-2-C<sup>14</sup> were obtained from the California Corporation for Biochemical Research, Los Angeles. In all cases, the supplied radioisotopes were diluted with carrier solutions to give the specific activities shown in the Results Section.

### Feeding Experiments

In all experiments, the plant materials were sliced to facilitate the penetration of the small amounts of radioactive materials supplied (Cossins and Beevers 1963). Bulky storage tissues such as carrot and radish roots were cut into cylinders 6 mm in diameter using a cork borer. Slices, 1 mm thick, were then cut from the cylinders using a razor blade. In experiments using young root tissues, the apical, 5 mm, were excised and used as experimental material. Before use in the feeding experiments, all plant materials were thoroughly washed in distilled water and blotted dry using paper towels. In the







TABLE 1

Plant tissues used in studies of Amino acid biosynthesis

| <u>Common Name<br/>of Plant</u> | <u>Genus, Species<br/>and Variety</u>                | <u>Tissue Examined</u>                 |
|---------------------------------|------------------------------------------------------|----------------------------------------|
| Radish                          | <u>Raphanus sativus</u><br>cv. 'Red boy short top'   | Leaves, storage<br>tissues, cotyledons |
| Carrot                          | <u>Daucus carota</u>                                 | Storage tissue                         |
| Castor Bean                     | <u>Ricinus communis</u> var.<br><u>zanzibarensis</u> | Endosperm, roots                       |
| Sunflower                       | <u>Helianthus annus</u><br>cv. 'Mammoth Russian'     | Cotyledons, roots                      |
| Corn                            | <u>Zea mays</u><br>cv. 'Falconer'                    | Roots, coleoptiles                     |
| Pea                             | <u>Pisum sativum</u><br>cv. 'Homesteader'            | Cotyledons, roots,<br>leaves, stem     |
| Pumpkin                         | <u>Cucurbita pepo</u><br>cv. 'Connecticut field'     | Cotyledons                             |
| Watermelon                      | <u>Citrullus vulgaris</u><br>cv. 'Early Canada'      | Cotyledons                             |
| Linseed                         | <u>Linum usitatissimum</u><br>cv. 'Redwing'          | Cotyledons                             |
| Oats                            | <u>Avena sativa</u><br>cv. 'Victory'                 | Roots                                  |
| Potato                          | <u>Solanum tuberosum</u>                             | Etiolated shoots                       |



majority of experiments, the plant materials were incubated in a Warburg flask (25 ml capacity) at 30° in air. Besides the plant material, the flasks contained small volumes of phosphate or tris-HCl buffer as indicated in the Results Section. The centre well of the flasks contained 0.2 ml of 20% KOH solution to absorb any carbon dioxide evolved during the experimental period. The absorbed carbon dioxide was converted to barium carbonate, plated onto glass fibre filter paper discs, and dried overnight at 100°. Carbon-14 content was assayed as described later and corrections for self absorption and background were made.

#### Analytical Methods

At the end of the experimental periods, the tissues were killed by boiling for 5 minutes in 20 mls of 80% ethanol. The tissues were then finely homogenized using a hand blender and centrifuged at 7,000 g for 15 minutes to separate the alcohol soluble fraction from insoluble residual material. The insoluble residue was then successively washed with 20 mls of 50% ethanol followed by 20 mls distilled water and the centrifugation repeated. The supernatants were combined and dried under reduced pressure at 40° using a flash evaporator. The dried material was washed with three 10 ml aliquots of anhydrous diethyl ether to remove lipid material.

The ether washed material was dissolved in 15 mls of distilled water at 30° and divided into four fractions using ion exchange resins (Canvin and Beevers 1961, Cossins and Beevers 1963).



## Ion Exchange Chromatography of Plant Extracts

In the present studies, the plant extracts were separated into three main fractions on the basis of ionic charge, using Dowex ion exchange resins.

1. Basic Compounds (positively charged), mainly amino acids.
2. Acidic Compounds (negatively charged), mainly organic acids, phosphate esters and nucleotides.
3. Neutral Compounds, mainly sugars.

The amino acid fraction was further separated into acidic amino acids and neutral and basic amino acids using Dowex resin in the acetate form.

## Ion Exchange Resins

Two types of resins were obtained from California Corporation for Biochemical Research, Los Angeles.

- (i) Cation exchange resin - Dowex 50W-X8 (Hydrogen form) 200 - 400 mesh.
- (ii) Anion exchange resin - Dowex 1-X10 (Chloride form) 200 - 400 mesh.

## Preparation of Anion Exchange Resin

The anion exchange resin, purchased in the chloride form, was converted to the acetate or formate forms before use. This was achieved by washing the chloride form of the resin in a large column with 1M sodium formate or sodium acetate solution until the effluent gave a negative chloride test. The resin was then washed with 0.1N formic acid or 0.1N acetic acid (10 mls/ 5 mls of resin). The resin was finally washed with distilled water until the effluent was neutral.





## Fractionation of Plant Extracts

Water soluble materials extracted from the plant tissues, as described earlier, were fractionated by allowing them to pass through a 4 X 1 cm column of Dowex (Hydrogen form) ion exchange resin. The effluent, containing the organic acids and sugars, was collected in an Erlenmeyer flask. The column was washed with 40 mls of distilled water to ensure complete removal of organic acids and sugars. The amino acids, which were adsorbed by this resin, were then eluted using 30 mls of 2N ammonium hydroxide solution. This treatment therefore separated the amino acids from the organic acids and sugars.

Separation of the organic acids from the sugars was next achieved using 3 X 1 cm columns of Dowex (formate) resin. Sugars passing through the column in the effluent, were completely removed from the resin using 40 mls of distilled water. The organic acids, which were adsorbed by the formate column were eluted with 30 mls of 4N formic acid.

The amino acids were next dissolved in 20 mls of distilled water and separated into neutral and basic amino acids and into acidic amino acids using Dowex resin in the acetate form. The amino acids, dissolved in water, were added to a 4 X 1 cm column of Dowex (acetate) resin. The neutral and basic amino acids were collected in the effluent from the column by washing with 40 mls of distilled water. The acidic amino acids (chiefly glutamic and aspartic acids) were eluted from the Dowex (acetate) resin column with 30 mls of 4N acetic acid.



## Chromatography of Amino Acids and Organic Acids

### 1. Amino Acids:

Components of the neutral and basic amino acid fraction were separated by two dimensional descending paper chromatography using 90% phenol:water (21:4 v/v) and n-butanol:acetic acid:water (4:1:5 v/v/v) as solvent systems and by one dimensional descending paper chromatography using n-propanol:ammonia (60:40 v/v). The radioactive areas on the two dimensional chromatograms were located by autoradiography using Kodak no-screen X-ray film. These areas were eluted and assayed for radioactivity (see later section). Aliquots of the eluates obtained from these areas were co-chromatographed with authentic amino acids detected by spraying with 0.5% ninhydrin in acetone.

In the above solvents, good separation of glycine and serine could not be achieved. These amino acids were therefore separated by a slight modification of the solvent used by Hardy, Holland and Naylor (1955). The modified solvent system used was n-butanol:acetone:water:diethylamine (40:40:20:6 by volume).

### 2. Organic Acids:

Further fractionation of the organic acid fraction, eluted from the Dowex (formate) resin, was achieved by gradient elution of the acids from 1 X 11 cm columns of Dowex resin (formate) by the method of Palmer (1955). The water reservoir contained 500mls of water having another reservoir above it containing 8N formic acid.



The water reservoir was connected to the 1 X 11 cm formate column (Dowex resin) and samples were eluted and collected using an automatic Buchler fraction collector. At a flow rate of 1 ml per minute, 120 tubes containing 3 mls of eluate were collected. An aliquot of each tube was plated on metal planchets, dried and assayed for radioactivity as described later.

The individual organic acids were identified by their position of elution from the Dowex-formate resin and by co-chromatography with authentic organic acids using n-propanol:ammonia (60:40 v/v), n-butanol:acetic acid:water (4:1:5 v/v/v), methanol:ammonia:water (90:5:5 v/v/v) and n-butanol:acetone:water:diethylamine (40:20:20:6 by volume) as solvent systems.

Glyoxylic acid was further identified by preparing 2,4-dinitrophenyl hydrazone of the fraction which was collected in tubes 5 to 9 (Turner and Quartley 1956). Identification of oxalate-C<sup>14</sup> was established by preparing the calcium salt (Millerd, Morton and Wells 1963). This was done by drying the material collected in tubes, 22-29, and treating it with an excess of calcium chloride in the presence of carrier oxalate. The white precipitate of calcium oxalate was washed with dilute acetic acid (pH 5.5), plated onto a fibre glass filter paper disc and mounted on a metal planchet for assay of carbon-14 content. The counts were corrected for self absorption and background.

#### Identification of Formate

In experiments where formate production was studied,







the plant tissues were killed by grinding in 20 mls of ice-cold 80% ammoniacal ethanol. Following centrifugation, the insoluble residue was washed successively with 30 mls of 50% ethanol and water. The supernatants containing the ammonium salts of organic acids were combined and dried under reduced pressure at 40°. The ammonium salts of the organic acids were then dissolved in 10 mls of distilled water and acidified by addition of 1 ml of ice-cold 88% phosphoric acid. After adjustment to 50 mls, the solution was distilled at 100°, the distillate being collected in 10 mls of ice-cold 0.1% NaOH. The distillate was concentrated to 5 mls and the carbon-14 content was assayed as described later. The presence of formate-C<sup>14</sup> in the distillate was demonstrated by co-chromatography with authentic formate-C<sup>14</sup> using n-propanol:ammonia (60:40 v/v), and n-butanol:acetone:water:diethylamine (40:40:20:6 by volume) as solvents. Further confirmation of formate was achieved by oxidation to carbon dioxide in the presence of mercuric ions (Tolbert, Clagett and Burris 1949).

#### Determination of C<sup>14</sup> in Insoluble Residue

After thorough drying at 100°, samples of the insoluble residue were subjected to acid hydrolysis by the method described by Ranjan and Lalorya (1960) or were combusted to carbon dioxide using the reagents of Van Slyke and Folch (1940) in a Stutz and Burris apparatus (Stutz and Burris 1951).

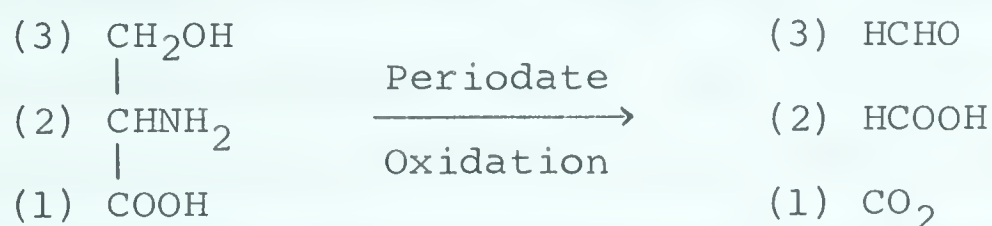
In experiments where the distribution of carbon-14 in protein amino acids was determined, the acid hydrolysate was filtered and dried under reduced pressure at 40°. The dried material was redissolved in 10 mls of distilled water and again



brought to dryness. The process was repeated until the hydrolysate was acid free. The acid-free hydrolysate was then dissolved in 10 mls of distilled water and the amino acids removed by ion exchange chromatography using Dowex resin in the hydrogen form (see above). Also, by paper chromatography, as mentioned earlier, these protein amino acids were separated and identified and assayed for carbon-14 activity.

#### Degradation of Serine-C<sup>14</sup>

Samples of serine-C<sup>14</sup> isolated in various experiments by the techniques described earlier, were degraded by periodate oxidation. This method yielded carbon dioxide from the carboxyl group and formate and formaldehyde from the 2 and 3 positions of serine respectively (Sakami 1950).



For recovery of the products of the periodate oxidation, the method of Sakami (1950) was modified as follows: A 2 ml sample of serine-C<sup>14</sup> was placed in the reaction flask of a Stutz and Burris apparatus together with 4 mls of 0.5M phosphate buffer (pH 5.2). Carbon dioxide-free air was passed through the apparatus for 10 minutes to ensure complete removal of carbon dioxide from the apparatus. Then 3 mls of 0.5M sodium periodate were introduced from the side arm and the carbon dioxide-free air passed through for a further hour. Carbon dioxide produced from the carboxyl group of serine was absorbed in a 15 X 0.5 cm





Vigreux column containing 10% KOH. Absorbed carbonate was converted to barium carbonate and assayed for radioactivity as described later. After removal of the carboxyl group as carbon dioxide, the reaction flask was rapidly cooled to 2° and the pH raised to 8 by addition of 1N NaOH solution. Formaldehyde derived from the 3 position of serine was then distilled off after addition of 1  $\mu$ mole of formaldehyde as carrier, and collected in 0.5% 2,4-dinitrophenyl hydrazine in 6N HCl. The 2,4-dinitrophenyl hydrazones of formaldehyde were extracted into 10% ethanol in chloroform (Turner and Quartley 1956) and radioactivity determined by plating 0.2 ml aliquots of the ethanol-chloroform extract on metal planchets.

After removal of the formaldehyde by distillation, the flask was cooled in ice and the contents adjusted to pH 2 by addition of 20% phosphoric acid. The acidified solution was distilled once again and formic acid, derived from the 2 position of serine, was collected in 2N NaOH solution. Samples (0.2 ml) of the formate solution were then plated on metal planchets followed by counting of radioactivity.

When samples of serine-C<sup>14</sup> were degraded by this procedure, recoveries of radioactivity from the respective carbon atoms were within the range of 90 - 95%.

#### Degradation of Glycine-C<sup>14</sup>

Samples of glycine-C<sup>14</sup> were degraded using ninhydrin in citrate buffer (Van Slyke, Dillon, MacFadyen and Hamilton 1941). This yielded carbon dioxide from the carboxyl group which was trapped in 20% (w/v) KOH, converted to BaCO<sub>3</sub> and assayed for radioactivity. In all cases, radioactivity of the





2 position of the glycine molecule was calculated by difference between the total radioactivity of the sample and radioactivity recovered as carbon dioxide when the sample was treated with ninhydrin.

### Enzyme Studies

Studies were conducted on the formic dehydrogenase and glyoxylate-glycine transaminase activity present in extracts prepared from tissues used in the feeding experiments. In both cases, attempts were made to determine whether the enzymes were intracellularly localized by separation of sub-cellular fractions. In addition, the glyoxylate-glycine transaminase system was partially purified.

### Formic Dehydrogenase

Extracts containing formic dehydrogenase activity were prepared from a variety of higher plant tissues as follows: Twenty grams fresh weight of tissue were chilled at 2° prior to grinding in a hand mortar. The grinding medium (20 mls) contained 0.02M  $MgCl_2$ , and 0.1M  $KH_2PO_4$  (pH 5.5) in 0.5M sucrose. Grinding was carried out at 5° and the crude homogenate was passed through four layers of cheese cloth. The homogenate was then centrifuged at 3000 g for 10 minutes at 5° to sediment cell debris and starch grains. The supernatant was again centrifuged at 23,000 g for 20 minutes at 5° to sediment the mitochondria. After removal of the high speed supernatant fraction, the mitochondrial pellet was resuspended in 10 mls of sucrose phosphate buffer (see above) and centrifuged again at 23,000 g for 20 minutes. The washed mitochondria were finally taken up in a small volume of sucrose-phosphate buffer



and the formic dehydrogenase activity assayed as described below:

One ml of enzyme was placed in a Warburg flask (25 ml capacity) and 0.1 ml of formate- $C^{14}$  solution (specific activity as indicated in Results Section) occupied the side arm. After equilibration at  $30^{\circ}$ , formate was tipped into the main compartment. The carbon dioxide, evolved from the enzymatic oxidation of formate, was absorbed in 0.2 ml of 10% KOH solution in the centre well of the flask. Absorbed carbonate was converted to  $BaCO_3$ , plated on filter paper discs, mounted on metal planchets and assayed for carbon-14 activity.

#### Glyoxylate-glycine Transaminase

A transaminase system, catalyzing the formation of glycine from glyoxylate and an amino donor, was extracted from a variety of plant tissues including carrot storage tissue, pea leaves, corn coleoptiles and sunflower cotyledons.

#### Preparation of Glyoxylate-glycine Transaminase from Carrot Tissues

A sample (20 g.f. wt.) of carrot storage tissue was ground finely with 20 mls of ice-cold 0.1M phosphate buffer (pH 7.5) in a hand blender. The homogenate was passed through four layers of cheese cloth and immediately centrifuged at 10,000 g for 20 minutes at  $-2^{\circ}$ . Following centrifugation, the supernatant was dialyzed against 0.1M phosphate buffer (pH 7.5) for 20 hours at  $5^{\circ}$ . The preparation was made up to volume and an aliquot of it was used for assaying transaminase activity as described below. Similar preparations of the enzyme were made from excised pea leaves and





corn coleoptiles. In germinating sunflower cotyledons where changes in the enzyme activity were followed from 1 to 5 days, only 10 g.f. wt. of tissues were used for enzyme preparation; the extraction procedure being that used for the carrot storage tissue.

#### Intracellular Localization of Glyoxylate-glycine Transaminase in Sunflower Cotyledons

Three day old sunflower cotyledons were chilled to 2° before grinding in ice-cold sucrose-phosphate buffer (pH 7.5). Mitochondrial and high speed supernatant fractions were separated by centrifugation as described earlier for formic dehydrogenase. The enzyme preparations were dialyzed against sucrose-phosphate buffer (pH 7.5) before assaying the enzyme activity.

#### Partial Purification of the Glyoxylate-glycine Transaminase of Sunflower Cotyledons

A sample of 3 day old sunflower cotyledons was homogenized in 100 mls of acetone at -10°. The acetone extract was discarded and powder dried thoroughly in a Vacuum dessicator maintained at 5° for 16 hours. Samples of the acetone powder were extracted in 0.1M phosphate buffer (pH 7.2) and centrifuged at 20,000 g for 20 minutes. The soluble protein was precipitated by additions of cold saturated ammonium sulphate solution from the supernatant. The precipitates obtained at 50%, 75% and 90% saturation with ammonium sulphate were centrifuged and redissolved in 0.1M phosphate buffer (pH 7.2). Aliquots of these preparations were then assayed for transaminase activity.





### Assaying of Glyoxylate-glycine Transaminase Activity

Glyoxylate-glycine transaminase activity was assayed by determining the rate of conversion of glyoxylate-1,2- $C^{14}$  into glycine- $C^{14}$ . One ml of the enzyme was placed in a Warburg flask and 0.1 ml of glyoxylate-1,2- $C^{14}$  (specific activities as indicated in Results Section) was added to the side arm. After equilibration for 5 minutes at  $30^{\circ}$ , air in the flasks was replaced by nitrogen and the glyoxylate- $C^{14}$  solution was tipped into the main compartment. At the end of the incubation period the transaminase reaction was stopped by addition of 10 mls of 80% boiling ethanol. The precipitated protein was removed by centrifugation and the supernatant analyzed for glycine- $C^{14}$  using ion exchange columns and paper chromatography as described in an earlier section.

### Determination of Protein Nitrogen

The protein nitrogen content of the enzyme preparations was determined by the micro-Kjeldahl method (Ballentine 1957).

### Assay of $C^{14}$ Content of Plant Extracts and Carbon Dioxide

Aliquots of the radioactive solutions extracted by the methods described were plated onto metal planchets. They were dried under an infra-red light in moving air, after adding 0.1 ml of absolute ethanol to ensure even spreading of the material. Carbon-14 content of the carbon dioxide produced in feeding experiments or in chemical degradations was absorbed in 20% KOH and converted to  $BaCO_3$  by addition of excess  $BaCl_2$  solution. The precipitate was filtered onto glass fibre filter paper discs. The discs were dried thoroughly at  $100^{\circ}$  and mounted on metal planchets. The planchets were assayed for



activity in a gas flow counter (Model C 110 B, Nuclear Chicago Corporation, Chicago, U.S.A.) with automatic sample changer and timer. The counts were corrected for self absorption and background.

#### Detection of $C^{14}$ Labelled Compounds on Paper Chromatograms

The one dimensional chromatograms were cut into strips 1-1½ inches wide and scanned for radioactivity using a 4 Pi Actigraph (Model 1036, Nuclear Chicago Corporation, Chicago, U.S.A.).



## RESULTS

### Production of Formate by Plant Tissues

There are reports in literature which indicate that formate or a 'formyl' radical can arise from glyoxylate, glyoxylate or glycine in experiments using leaf tissues (Tolbert, Clagett and Burris 1949, Wang and Burris 1963). As formate can be utilized as a 'formyl' radical in animal tissues for the biosynthesis of serine and methionine (Sakami 1955, Welch and Sakami 1950), in plants, the production of formate might represent an important metabolic reaction where methionine and serine biosynthesis is occurring.

Experiments were therefore designed to determine whether formate might arise from glycine or glyoxylate. Tissues used in these studies included germinating sunflower cotyledons which produce glyoxylate from isocitrate (Bradbeer and Stumpf 1959) and carrot storage tissues which synthesize the  $\beta$ -C serine from methanol (Cossins and Beevers 1962). In the experiments summarized in Table 2, slices of the plant tissues were incubated with equal specific activities of glyoxylate-1, 2- $C^{14}$  and glycine-2 $C^{14}$ .

In both tissues, glyoxylate-1, 2- $C^{14}$  produced more formate- $C^{14}$  as compared to glycine- $C^{14}$  though the specific activity of glyoxylate- $C^{14}$  and glycine- $C^{14}$  was the same. Furthermore, formate possibly arises from the two position of glyoxylate or glycine. Since glycine was exclusively labelled in the 2 position, it should have produced more formate- $C^{14}$  if production of formate from glyoxylate and glycine is equally





TABLE 2

The Production of formate from glyoxylate-1,2-Cl<sup>14</sup> and glycine-2-Cl<sup>14</sup> by Sunflower cotyledons and Carrot storage tissue

0.5 g slices of tissues incubated at 30° for 1 hr. with 100 μmoles of phosphate buffer (pH 5.6) and 1 μmole of glyoxylate or glycine containing 2 μc of Cl<sup>14</sup>. The tissues were killed with ice cold ammonical 80% ethanol. Formate-Cl<sup>14</sup> was determined as described in the Methods Section.

| <u>Treatment</u>                                    | <u>Cl<sup>14</sup> activity in formate (cpm)</u> |                              |
|-----------------------------------------------------|--------------------------------------------------|------------------------------|
|                                                     | <u>Sunflower cotyledons*</u>                     | <u>Carrot storage tissue</u> |
| Glyoxylate-1,2-Cl <sup>14</sup>                     | 33,200                                           | 32,000                       |
| Glyoxylate-1,2-Cl <sup>14</sup> + 10 μmoles formate | 28,300                                           | 70,000                       |
| Glycine-2-Cl <sup>14</sup>                          | 7,100                                            | 3,200                        |

\* 3 day old



efficient. Therefore it can be concluded that glyoxylate is a relatively better precursor for the formate production as compared to glycine.

#### Utilization of Formate-C<sup>14</sup> by Plant Tissues

As mentioned in an earlier section, formate is known to be produced in various plant tissues. In the present investigation also, evidence for its production was obtained from sunflower cotyledons and carrot storage tissue. Work with animal tissues has shown that formate is used in the biosynthesis of serine in the presence of acceptor glycine (Sakami 1955, Alexander and Greenberg 1955). Therefore, a study was made on the metabolism of exogenously supplied formate-C<sup>14</sup> with a particular emphasis on amino acid biosynthesis.

#### Germinating Seeds

The metabolism of formate-C<sup>14</sup> by slices of pea cotyledons and the endosperm of castor bean seeds is shown in Table 3. In both cases, carbon dioxide was the major product of formate metabolism. However, in addition, large amounts of radioactivity were detected in the neutral and basic amino acid fraction. In castor bean endosperm slices, formate-C<sup>14</sup> was also extensively incorporated into the organic acids, the sugar fraction and the acidic amino acids.

The ability to utilize formate-C<sup>14</sup> was obvious during the early stages of germination of castor bean seeds rising to a maximum in 3 day old seedlings (Table 4). During the first 5 days of germination, the endosperm converted the formate-C<sup>14</sup> into all the fractions isolated. Carbon dioxide was the chief



TABLE 3

The Utilization of formate-Cl<sup>14</sup> by germinating seedlings

1 g of slices of castor bean endosperm (5 day old) and pea cotyledons (3 day old) incubated in air at 300 for 3 hr. in darkness with 200  $\mu$ moles of tris buffer (pH 7.4) and 1  $\mu$ mole of formate containing 5  $\mu$ c of Cl<sup>14</sup>

| <u>Fraction</u>                     | <u>Castor bean endosperm</u> |                                          | <u>Pea cotyledons</u>        |                                          |
|-------------------------------------|------------------------------|------------------------------------------|------------------------------|------------------------------------------|
|                                     | <u>Cl<sup>14</sup> (cpm)</u> | <u>% of incorporated Cl<sup>14</sup></u> | <u>Cl<sup>14</sup> (cpm)</u> | <u>% of incorporated Cl<sup>14</sup></u> |
| Carbon Dioxide                      | 3,013,000                    | 76.9                                     | 2,256,000                    | 89.4                                     |
| Lipids                              | 32,000                       | 0.8                                      | 2,000                        | 0.08                                     |
| Organic Acids                       | 51,000                       | 1.3                                      | 7,000                        | 0.27                                     |
| Sugars                              | 84,000                       | 2.1                                      | 9,600                        | 0.38                                     |
| Amino Acids                         |                              |                                          |                              |                                          |
| Neutral & Basic amino acids         | 396,000                      | 10.1                                     | 224,000                      | 8.9                                      |
| Acidic amino acids                  | 240,000                      | 6.3                                      | 18,400                       | 0.73                                     |
| Residue                             | 96,000                       | 2.4                                      | 2,000                        | 0.08                                     |
| Total Cl <sup>14</sup> incorporated | 3,918,000                    |                                          | 2,519,000                    |                                          |





TABLE 4

The Utilization of formate-C<sup>14</sup> during germination of Castor bean seeds

| Fraction                              | 1 day old                |                                    | 2 day old                |                                    | 3 day old                |                                    | 4 day old                |                                    | 5 day old                |                                    |
|---------------------------------------|--------------------------|------------------------------------|--------------------------|------------------------------------|--------------------------|------------------------------------|--------------------------|------------------------------------|--------------------------|------------------------------------|
|                                       | C <sup>14</sup><br>(cpm) | % of<br>incorp.<br>C <sup>14</sup> | C <sup>14</sup><br>(cpm) | % of<br>incorp.<br>C <sup>14</sup> | C <sup>14</sup><br>(cpm) | % of<br>incorp.<br>C <sup>14</sup> | C <sup>14</sup><br>(cpm) | % of<br>incorp.<br>C <sup>14</sup> | C <sup>14</sup><br>(cpm) | % of<br>incorp.<br>C <sup>14</sup> |
| Carbon Dioxide                        | 31,000                   | 31.7                               | 167,000                  | 67.7                               | 208,000                  | 56.0                               | 185,000                  | 61.7                               | 234,000                  | 66.7                               |
| Lipids                                | 2,000                    | 2.0                                | 3,300                    | 1.4                                | 6,000                    | 1.6                                | 2,500                    | 0.8                                | 800                      | 0.2                                |
| Organic Acids                         | 23,000                   | 23.5                               | 7,500                    | 3.0                                | 32,000                   | 8.6                                | 23,400                   | 7.8                                | 23,400                   | 6.6                                |
| Sugars                                | 2,000                    | 2.0                                | 12,000                   | 5.0                                | 15,000                   | 4.0                                | 9,600                    | 3.2                                | 21,400                   | 6.1                                |
| Amino Acids                           |                          |                                    |                          |                                    |                          |                                    |                          |                                    |                          |                                    |
| Neutral & Basic<br>amino acids        | 25,800                   | 26.4                               | 41,000                   | 16.6                               | 68,400                   | 18.4                               | 52,700                   | 17.6                               | 55,700                   | 15.9                               |
| Acidic amino acids                    | 8,200                    | 8.4                                | 8,200                    | 3.4                                | 23,000                   | 6.2                                | 14,500                   | 4.8                                | 7,400                    | 2.1                                |
| Residue                               | 5,800                    | 6.0                                | 7,500                    | 3.0                                | 18,800                   | 5.1                                | 12,300                   | 4.1                                | 7,800                    | 2.2                                |
| Total C <sup>14</sup><br>incorporated | 97,800                   |                                    | 246,500                  |                                    | 371,200                  |                                    | 300,000                  |                                    | 350,500                  |                                    |



product of formate metabolism but the total  $C^{14}$  utilized varied as germination proceeded. The endosperm of 1 day old seedlings incorporated large amounts of the label into organic acids and into neutral and basic amino acids. The percentage of carbon-14 entering the sugar fraction fluctuated, rising to a maximum in the 5 day old seedlings.

#### Corn Coleoptiles and Potato Shoots

Slices of these tissues were incubated with formate- $C^{14}$  as indicated in Table 5. As in germinating seeds, carbon dioxide was a major product of formate metabolism. In potato shoots, this fraction contained 83% of the carbon-14 incorporated. In both cases, the neutral and basic amino acids were important products and in corn coleoptiles, there was a striking incorporation of the label into the sugar fraction.

#### Carrot Tissues

Formate metabolism in these tissues was examined in a 3 hour experiment (Table 6). Carbon dioxide was again the chief product of formate metabolism comprising over 65% of the carbon-14 incorporated. Large amounts of radioactivity were also detected in the neutral and basic amino acids, in the acidic amino acids and in the residue.

In another experiment (Table 7), the sequence of incorporation of the label into the products of formate metabolism was investigated. After 5 minutes of formate metabolism, carbon dioxide and organic acids contained the bulk of the carbon-14 incorporated. With increase in the period of feeding, the amounts of  $C^{14}O_2$  increased rapidly whereas the percentage of carbon-14 in the organic acids greatly decreased.



TABLE 5

The Utilization of formate-Cl<sup>4</sup> by Corn coleoptiles and Potato shoots

1 g of slices of tissues incubated in air at 30° for 3 hr. in darkness with 200  $\mu$ moles of tris buffer (pH 7.4) and 1  $\mu$ mole of formate containing 5  $\mu$ c of Cl<sup>4</sup>.

| Fraction                           | Corn coleoptiles      |                                   | Potato shoots         |                                   |
|------------------------------------|-----------------------|-----------------------------------|-----------------------|-----------------------------------|
|                                    | Cl <sup>4</sup> (cpm) | % of incorporated Cl <sup>4</sup> | Cl <sup>4</sup> (cpm) | % of incorporated Cl <sup>4</sup> |
| Carbon Dioxide                     | 517,700               | 38.0                              | 2,400,000             | 82.9                              |
| Lipids                             | 4,800                 | 0.35                              | 18,400                | 0.63                              |
| Organic Acids                      | 64,000                | 4.7                               | 40,000                | 1.4                               |
| Sugars                             | 404,800               | 29.7                              | 21,600                | 0.74                              |
| Amino Acids                        |                       |                                   |                       |                                   |
| Neutral & Basic amino acids        | 221,000               | 16.2                              | 332,800               | 11.5                              |
| Acidic amino acids                 | 41,600                | 3.0                               | 70,400                | 2.4                               |
| Residue                            | 108,000               | 8.0                               | 12,000                | 0.4                               |
| Total Cl <sup>4</sup> incorporated | 1,362,000             |                                   | 2,895,200             |                                   |





TABLE 6

The Utilization of formate-C<sup>14</sup> by Carrot slices

1 g of carrot storage tissue incubated in air at 30° for 3 hr. in darkness with 200  $\mu$ moles of tris buffer (pH 7.4) and 1  $\mu$ mole of formate containing 5  $\mu$ c of C<sup>14</sup>.

| <u>Fraction</u>                    | <u>C<sup>14</sup> (cpm)</u> | <u>% of incorporated C<sup>14</sup></u> |
|------------------------------------|-----------------------------|-----------------------------------------|
| Carbon Dioxide                     | 730,000                     | 65.5                                    |
| Lipids                             | 2,000                       | 0.2                                     |
| Organic Acids                      | 45,600                      | 4.1                                     |
| Sugars                             | 8,800                       | 0.8                                     |
| Amino Acids                        |                             |                                         |
| Neutral & Basic<br>amino acids     | 179,200                     | 16.1                                    |
| Acidic amino acids                 | 80,000                      | 7.2                                     |
| Residue                            | 66,400                      | 6.0                                     |
| Total C <sup>14</sup> incorporated | 1,112,000                   |                                         |



TABLE 7

The Sequence of incorporation of  $\text{Cl}^{14}$  from formate- $\text{Cl}^{14}$  by Carrot slices

1 g of slices of carrot storage tissue incubated in air at  $30^{\circ}$  in darkness with 200  $\mu$ moles of tris buffer (pH 7.4) and 1  $\mu$ mole of formate containing 5  $\mu$ c of  $\text{Cl}^{14}$ .

| Fraction                            | 5 mins.                   |                                     | 10 mins.                  |                                     | 20 mins.                  |                                     | 30 mins.                  |                                     |
|-------------------------------------|---------------------------|-------------------------------------|---------------------------|-------------------------------------|---------------------------|-------------------------------------|---------------------------|-------------------------------------|
|                                     | $\text{Cl}^{14}$<br>(cpm) | % of<br>$\text{Cl}^{14}$<br>incorp. | $\text{Cl}^{14}$<br>(cpm) | % of<br>$\text{Cl}^{14}$<br>incorp. | $\text{Cl}^{14}$<br>(cpm) | % of<br>$\text{Cl}^{14}$<br>incorp. | $\text{Cl}^{14}$<br>(cpm) | % of<br>$\text{Cl}^{14}$<br>incorp. |
| Carbon Dioxide                      | 16,800                    | 58.0                                | 104,000                   | 78.8                                | 360,000                   | 83.2                                | 596,000                   | 82.8                                |
| Organic Acids                       | 6,300                     | 21.1                                | 9,000                     | 6.8                                 | 17,600                    | 4.1                                 | 32,800                    | 4.5                                 |
| Sugars                              | 200                       | 0.7                                 | 300                       | 0.2                                 | 1,600                     | 0.37                                | 1,200                     | 0.16                                |
| Amino Acids                         |                           |                                     |                           |                                     |                           |                                     |                           |                                     |
| Neutral & Basic<br>amino acids      | 600                       | 2.0                                 | 800                       | 0.6                                 | 2,800                     | 0.65                                | 5,000                     | 0.7                                 |
| Acidic amino acids                  | 2,000                     | 6.9                                 | 8,800                     | 6.7                                 | 34,000                    | 8.0                                 | 62,000                    | 8.3                                 |
| Residue                             | 3,000                     | 10.3                                | 9,000                     | 6.8                                 | 14,000                    | 3.2                                 | 23,000                    | 3.2                                 |
| Total $\text{Cl}^{14}$ incorporated | 28,900                    |                                     | 131,900                   |                                     | 430,000                   |                                     | 720,000                   |                                     |



### Radish Tissues

The mature green leaves and the storage tissues of radishes were incubated with formate-C<sup>14</sup> in the dark as shown in Table 8. Considerably more formate-C<sup>14</sup> was utilized by the leaves during the experimental period. In both tissues, however, carbon dioxide contained the largest amount of radioactivity. In the storage tissue, the organic acids and acidic amino acids were heavily labelled. Only small amounts of radioactivity were detected in the neutral and basic amino acids, in the sugars and in the insoluble residue. Formate-C<sup>14</sup> metabolism by the radish leaves resulted in radioactivity being distributed in all of the fractions isolated. In particular, the organic acids and neutral and basic amino acids were important products of this metabolism.

### Excised Roots

The root tips (0.5 cm) of a variety of 3 day old seedlings were removed and incubated with formate-C<sup>14</sup> as shown in Tables 9 and 10. In all cases, large amounts of formate-C<sup>14</sup> were converted to carbon dioxide. The organic acids were the next most heavily labelled fraction with the exception of the sunflower roots where acidic amino acids incorporated more activity. Furthermore, formate was incorporated into the neutral and basic amino acids to a considerable extent; in pea roots this fraction accounted for 19 percent of the radioactivity incorporated.

### The Effect of Illumination on Formate Metabolism

Doman and Romanova (1962) have recently suggested, that in certain leaves, formate-C<sup>14</sup> was converted to carbon





TABLE 8

The Utilization of formate-Cl<sup>14</sup> by Radish tissues

0.5 g of slices of tissues incubated in air at 300 for 3 hr. in darkness with 200  $\mu$ moles of tris buffer (pH 7.4) and 1  $\mu$ mole of formate containing 5  $\mu$ c of Cl<sup>14</sup>.

| Fraction                            | Storage tissue*        |                                    | Leaves**               |                                    |
|-------------------------------------|------------------------|------------------------------------|------------------------|------------------------------------|
|                                     | Cl <sup>14</sup> (cpm) | % of incorporated Cl <sup>14</sup> | Cl <sup>14</sup> (cpm) | % of incorporated Cl <sup>14</sup> |
| Carbon Dioxide                      | 137,600                | 70.0                               | 830,400                | 83.6                               |
| Lipids                              | 3,000                  | 1.5                                | 10,700                 | 1.7                                |
| Organic Acids                       | 22,600                 | 11.5                               | 33,900                 | 3.4                                |
| Sugars                              | 1,600                  | 0.8                                | 6,700                  | 0.7                                |
| Amino Acids                         |                        |                                    |                        |                                    |
| Neutral & Basic amino acids         | 4,800                  | 2.4                                | 22,900                 | 2.3                                |
| Acidic amino acids                  | 18,300                 | 9.3                                | 14,400                 | 1.4                                |
| Residue                             | 9,200                  | 4.7                                | 72,400                 | 7.3                                |
| Total Cl <sup>14</sup> incorporated | 196,800                |                                    | 992,400                |                                    |

\* root storage tissue obtained from commercial sources

\*\* excised from 3 week old seedlings



TABLE 9

The Utilization of formate-Cl<sup>4</sup> by excised Root tips

1 g of root tips incubated at 300 for 15 mins. with 300  $\mu$ moles of tris buffer (pH 7.4) and 1  $\mu$ mole of formate containing 5  $\mu$ c of Cl<sup>4</sup>.

| Fraction                           | Corn*                    |                                    | Pea**                    |                                    | Castor bean**            |                                    | Oats**                   |                                    |
|------------------------------------|--------------------------|------------------------------------|--------------------------|------------------------------------|--------------------------|------------------------------------|--------------------------|------------------------------------|
|                                    | Cl <sup>4</sup><br>(cpm) | % of<br>incorp.<br>Cl <sup>4</sup> | Cl <sup>4</sup><br>(cpm) | % of<br>incorp.<br>Cl <sup>4</sup> | Cl <sup>4</sup><br>(cpm) | % of<br>incorp.<br>Cl <sup>4</sup> | Cl <sup>4</sup><br>(cpm) | % of<br>incorp.<br>Cl <sup>4</sup> |
| Carbon Dioxide                     | 41,600                   | 73.1                               | 24,000                   | 45.4                               | 135,200                  | 89.0                               | 64,000                   | 80.3                               |
| Organic Acids                      | 5,600                    | 9.8                                | 12,500                   | 23.6                               | 7,200                    | 4.7                                | 6,000                    | 7.5                                |
| Sugars                             | 1,600                    | 2.8                                | 2,300                    | 4.3                                | 1,200                    | 0.8                                | 1,000                    | 1.3                                |
| Amino Acids                        |                          |                                    |                          |                                    |                          |                                    |                          |                                    |
| Neutral & Basic<br>amino acids     | 4,300                    | 7.5                                | 10,000                   | 19.0                               | 6,100                    | 4.0                                | 3,000                    | 3.8                                |
| Acidic amino acids                 | ...                      | ...                                | ...                      | ...                                | 800                      | 0.5                                | ...                      | ...                                |
| Residue                            | 3,800                    | 6.7                                | 4,000                    | 7.6                                | 1,300                    | 0.8                                | 5,700                    | 7.1                                |
| Total Cl <sup>4</sup> incorporated | 56,900                   |                                    | 52,800                   |                                    | 151,800                  |                                    | 79,700                   |                                    |

\* excised from 3 day old seedlings

\*\* excised from 4 day old seedlings



TABLE 10

The Utilization of formate-Cl4 by excised root tips of Sunflower seedlings

1 g of excised root tips from 3 day old seedlings incubated at 30° for 15 mins. and 1 hr. with 300  $\mu$ moles of tris buffer (pH 7.4) and 1  $\mu$ mole of formate containing 5  $\mu$ c of Cl4.

| <u>Fraction</u>             | <u>15 mins.</u>  |                              | <u>1 hr.</u>     |                              |
|-----------------------------|------------------|------------------------------|------------------|------------------------------|
|                             | <u>Cl4 (cpm)</u> | <u>% of incorporated Cl4</u> | <u>Cl4 (cpm)</u> | <u>% of incorporated Cl4</u> |
| Carbon Dioxide              | 35,600           | 38.7                         | 248,000          | 67.3                         |
| Organic Acids               | 13,200           | 14.3                         | 10,400           | 2.8                          |
| Sugars                      | 2,400            | 2.6                          | 8,000            | 2.1                          |
| Amino Acids                 |                  |                              |                  |                              |
| Neutral & Basic amino acids | 8,300            | 9.0                          | 21,500           | 5.9                          |
| Acidic amino acids          | 22,500           | 24.4                         | 51,600           | 14.0                         |
| Residue                     | 10,000           | 10.9                         | 29,000           | 8.0                          |
| Total Cl4 incorporated      | 92,000           |                              | 368,500          |                              |





dioxide which was then assimilated by photosynthesis. This pathway was the major one for the incorporation of formate- $C^{14}$  and consequently in darkness, formate metabolism proceeded at reduced rates. In the present studies, mature pea leaves and stems were incubated with formate- $C^{14}$  (Table 11) in the light and dark, in order to ascertain whether photo-assimilation of formate was important in these tissues.

In both tissues, greater amounts of formate were utilized in the dark. In all cases, carbon dioxide was a major product, the percentage of carbon- $^{14}$  incorporated into this fraction being slightly reduced by illumination. In both tissues, illumination resulted in considerable increases in the amounts of formate- $C^{14}$  being incorporated into the sugar and neutral and basic amino acid fractions.

#### Comparison Between Incorporation Patterns of Carbonate- $C^{14}$ and Formate- $C^{14}$ in Corn Coleoptiles

Several reports in literature (Bidwell and Ghosh 1963, Doman and Romanova 1962) have suggested that formate is oxidized to carbon dioxide prior to its incorporation into cellular components. This possibility was examined by comparing the patterns of carbon- $^{14}$  incorporation following formate- $C^{14}$  and carbonate- $C^{14}$  feeding (Table 12). The corn coleoptiles readily incorporated radioactivity from supplied carbonate- $C^{14}$  into the components of the alcohol soluble fraction. Of this incorporation, 39% was in the organic acids and 47% in the amino acids. In contrast, formate- $C^{14}$  was incorporated to a lesser extent into the organic acids (26%), and to a greater extent (62%) into the amino acids. When



TABLE 11

The Effect of illumination on the utilization of formate-Cl<sup>14</sup> by Pea seedling tissues

0.25 g of pea leaves and stems excised from 15 day old seedlings incubated at 30° for 1 hr. with 100  $\mu$ moles of phosphate buffer (pH 5.5) and 1  $\mu$ mole of formate containing 2  $\mu$ c of Cl<sup>14</sup>. Illuminated tissues exposed to 400 ft-c during experimental period.

| Fraction                       | Leaves                      |                        |                          | Stems                  |                             |                          |                        |
|--------------------------------|-----------------------------|------------------------|--------------------------|------------------------|-----------------------------|--------------------------|------------------------|
|                                | Illuminated<br>Cl4<br>(cpm) | % of<br>incorp.<br>Cl4 | Darkened<br>Cl4<br>(cpm) | % of<br>incorp.<br>Cl4 | Illuminated<br>Cl4<br>(cpm) | Darkened<br>Cl4<br>(cpm) | % of<br>incorp.<br>Cl4 |
| Carbon Dioxide                 | 131,900                     | 78.5                   | 224,000                  | 88.0                   | 76,500                      | 108,300                  | 84.9                   |
| Lipids                         | 1,600                       | 0.9                    | 2,500                    | 0.9                    | 6,000                       | 1,500                    | 1.2                    |
| Organic Acids                  | 6,000                       | 3.6                    | 7,400                    | 2.9                    | 10,700                      | 8,400                    | 6.6                    |
| Sugars                         | 8,600                       | 5.1                    | 1,500                    | 0.59                   | 800                         | 300                      | 0.2                    |
| Amino Acids                    |                             |                        |                          |                        |                             |                          |                        |
| Neutral & Basic<br>amino acids | 13,000                      | 7.7                    | 8,400                    | 3.3                    | 9,600                       | 3,000                    | 2.3                    |
| Acidic amino acids             | 6,800                       | 4.0                    | 10,100                   | 4.0                    | 11,800                      | 5,700                    | 4.5                    |
| Residue                        | 1,000                       | 0.6                    | 6,000                    | 0.2                    | 1,400                       | 300                      | 0.2                    |
| Total Cl4 incorporated         | 168,000                     |                        | 254,500                  |                        | 111,400                     | 127,500                  |                        |



TABLE 12

Incorporation of C14 from carbonate-C14 and formate-C14 by Corn coleoptiles, Ethanol Soluble Fraction

0.5 g of excised coleoptiles (4 day old) incubated in darkness at 300 for 6 hr. with 100  $\mu$ moles tris buffer (pH 7.2) and 1  $\mu$ mole of carbonate containing 5  $\mu$ c of C14 or formate containing 2  $\mu$ c of C14 as indicated.

| Fraction                   | Carbonate-C14 |                       | Formate-C14 |                       | Formate-C14 + 10 $\mu$ moles of carbonate |                       |
|----------------------------|---------------|-----------------------|-------------|-----------------------|-------------------------------------------|-----------------------|
|                            | C14 (cpm)     | % of incorporated C14 | C14 (cpm)   | % of incorporated C14 | C14 (cpm)                                 | % of incorporated C14 |
| Lipids                     | 1,000         | 5                     | 3,300       | 7                     | 1,900                                     | 7                     |
| Organic Acids              | 7,400         | 39                    | 12,900      | 26                    | 7,400                                     | 27                    |
| Sugars                     | 1,400         | 8                     | 2,400       | 5                     | 3,000                                     | 11                    |
| Amino Acids                | 8,750         | 47                    | 31,500      | 62                    | 15,100                                    | 55                    |
| Total C14 incorporated     | 18,550        |                       | 50,100      |                       | 27,400                                    |                       |
| (ethanol soluble fraction) |               |                       |             |                       |                                           |                       |







formate-C<sup>14</sup> solution was supplied in the presence of added carbonate, the total amount of carbon-14 entering the ethanol soluble fraction was considerably reduced.

#### Production of Amino Acids from Formate-C<sup>14</sup>

Besides the major conversion of formate-C<sup>14</sup> to carbon dioxide, all the tissues examined incorporated considerable amounts of formate carbon into the neutral and basic amino acids. The amino acids in this fraction were separated by paper chromatography. The carbon-14 contents of the separated amino acids are shown in Table 13. In all tissues, serine, alanine and methionine sulphoxide contained the bulk of the radioactivity present in this fraction. Serine alone accounted for 74.9% of the carbon-14 content of the neutral and basic amino acid fraction in carrot tissues, 50% in corn coleoptiles and 54.9% in potato shoots. Incorporation of formate-C<sup>14</sup> into serine has been reported in barley leaves by Tolbert (1955) and in wheat by McConnell and Bilinski (1959). Methionine sulphoxide, a common oxidation product of methionine, was labelled together with methionine in all these tissues. These findings might therefore suggest that formate-C<sup>14</sup> was utilized as a 'formyl' radical for the biosynthesis of serine and methionine by a pathway involving a transmethylation reaction similar to that demonstrated in animal tissues.

#### Intramolecular Distribution of C<sup>14</sup> in Serine

It has been suggested by Alexander and Greenberg (1955) and Sakami (1955) that serine can be produced from glycine and a 'formyl' radical by animal tissues. In this pathway, carbons 1 and 2 of the serine molecule are derived from the corresponding glycine carbons and the 3 position is derived from the



TABLE 13

The Utilization of formate-Cl4 by plant tissues, neutral and basic amino acid fraction

Experimental procedure as in tables 3, 5 and 6.

| Amino Acid                                                          | Carrot     |                         | Castor Bean<br>Endosperm |                         | Corn Coleoptiles |                         | Potato Shoots |                         |
|---------------------------------------------------------------------|------------|-------------------------|--------------------------|-------------------------|------------------|-------------------------|---------------|-------------------------|
|                                                                     | Cl4 (cpm)  | % of<br>incorp.<br>Cl4* | Cl4 (cpm)                | % of<br>incorp.<br>Cl4* | Cl4 (cpm)        | % of<br>incorp.<br>Cl4* | Cl4 (cpm)     | % of<br>incorp.<br>Cl4* |
| Serine                                                              | 134,000    | 74.9                    | 107,800                  | 27.2                    | 110,500          | 50.0                    | 181,000       | 54.4                    |
| Alanine                                                             | 6,700      | 3.7                     | 40,000                   | 10.1                    | 18,500           | 8.4                     | 84,800        | 25.5                    |
| Methionine                                                          | 4,800      | 2.7                     | 25,400                   | 6.4                     | 46,000           | 20.8                    | 15,800        | 4.7                     |
| Methionine sulphoxide                                               | 28,700     | 16.0                    | 162,000                  | 40.9                    | 34,500           | 15.6                    | not active    | -                       |
| Histidine                                                           | not active | -                       | 30,400                   | 7.7                     | not active       | -                       | 51,200        | 15.4                    |
| Others                                                              | 4,800      | 2.7                     | 30,400                   | 7.7                     | 11,500           | 5.2                     | not active    | -                       |
| Total Cl4 incorporated in<br>neutral & basic amino<br>acid fraction | 179,000    |                         | 396,000                  |                         | 221,000          |                         | 332,800       |                         |

\* expressed as % of total Cl4 incorporated into the neutral and basic amino acid fraction



'formyl' radical. Clearly, if this pathway is operating in the plant tissues used in the present studies, formate-C<sup>14</sup> might be converted to a 'formyl-C<sup>14</sup>' radical and condensed with glycine to produce serine-C<sup>14</sup>. Furthermore, the carbon-14 present in the serine produced should be predominantly in the 3 position.

In order to test this hypothesis, serine-C<sup>14</sup>, isolated from carrot, potato shoots and corn coleoptiles, was degraded to determine the intramolecular distribution of carbon-14. The results are shown in Table 14. In all cases, the 3 position accounted for 58.0 - 60.0% of the total radioactivity present in the molecule. The 2 position contained 36 - 40% and the 1 position only 2 - 3.2% of the total carbon-14 content. The results of these degradation studies therefore indicate that formate-C<sup>14</sup> was mainly incorporated into the 3 position of the serine molecule. This might suggest that formate-C<sup>14</sup>, as a 'formyl' radical condensed with endogenous glycine in a trans-methylation reaction producing serine. Labelling of the 1 and 2 positions of the serine molecule might also indicate that the glycine pool involved in this serine biosynthesis was itself labelled with carbon-14.





TABLE 14

The Intramolecular distribution of  $C^{14}$  in Serine following formate- $C^{14}$  feeding to plant tissues

Samples of Serine- $C^{14}$  recovered from tissues after 3 hr. formate- $C^{14}$  metabolism were degraded using periodate (see Methods). Expressed as percentage of  $C^{14}$  recovered from products of degradation procedures.

|                    | <u>Carbon Number</u> | <u>Carrot</u> | <u>Potato Shoots</u> | <u>Corn Coleoptiles</u> |
|--------------------|----------------------|---------------|----------------------|-------------------------|
| COOH               | 1                    | 2.0           | 2.2                  | 3.2                     |
|                    |                      |               |                      |                         |
| CHNH <sub>2</sub>  | 2                    | 40.0          | 39.6                 | 36.2                    |
|                    |                      |               |                      |                         |
| CH <sub>2</sub> OH | 3                    | 58.0          | 58.2                 | 60.6                    |



## The Effect of Homocysteine and Glycine on Formate-C<sup>14</sup> Metabolism

Studies on radiosulphate utilization in higher plant tissues have indicated that cysteine and cysteic acid are the first compounds to be labelled in 15 minutes. With increase in the experimental period to 60 minutes, S<sup>35</sup>O<sub>4</sub> was incorporated into taurine, homocysteine and methionine (Sinha and Cossins 1963). It is therefore possible that cysteine might be converted to methionine via homocysteine by reversal of the pathway described by Tabachnick and Tarver (1955) for the conversion of methionine to cysteine in rats. The synthesis of methionine from homocysteine is known to involve a trans-methylation reaction in animal tissues (Cantoni 1952). It is therefore possible that additions of homocysteine might greatly increase the metabolism of formate-C<sup>14</sup> by stimulating this transmethylation reaction. Similarly, additions of glycine might stimulate the transmethylation reaction leading to serine formation. Experiments were therefore designed to test this hypothesis using radish cotyledons and pea leaves.

### Radish Cotyledons

Slices of fifteen-day old radish cotyledons were supplied with formate-C<sup>14</sup> for three hours in the presence of micromolar amounts of homocysteine and glycine in air and in nitrogen. After the experimental period, the neutral and basic amino acid fraction was separated as described in the Materials and Methods Section. In air, additions of homocysteine enhanced the incorporation of carbon-14 from formate-C<sup>14</sup> 3.5 times into the neutral and basic amino acid fraction (Table 15, 16). Chromatographic analysis of the amino acids present in



TABLE 15

The Effect of homocysteine and glycine on the metabolism of formate-Cl<sup>14</sup> by Radish cotyledons in air, neutral and basic amino acid fraction

0.5 g slices of cotyledons incubated in air at 30° for 3 hr. in light (400 f.c.) with 300  $\mu$ moles tris buffer (pH 7.4) and 1  $\mu$ mole of formate containing 10  $\mu$ c of Cl<sup>14</sup>.

| Amino Acids                         | formate-Cl <sup>14</sup> : control |                                       | formate-Cl <sup>14</sup> +<br>4 $\mu$ moles homocysteine |                                       | formate-Cl <sup>14</sup> +<br>4 $\mu$ moles glycine |                                       |
|-------------------------------------|------------------------------------|---------------------------------------|----------------------------------------------------------|---------------------------------------|-----------------------------------------------------|---------------------------------------|
|                                     | Cl <sup>14</sup> (cpm)             | % of Cl <sup>14</sup><br>incorporated | Cl <sup>14</sup> (cpm)                                   | % of Cl <sup>14</sup><br>incorporated | Cl <sup>14</sup> (cpm)                              | % of Cl <sup>14</sup><br>incorporated |
| Glycine                             | 17,500                             | 33.7                                  | 12,400                                                   | 6.8                                   | 3,000                                               | 6.0                                   |
| Alanine                             | -                                  | -                                     | 17,000                                                   | 9.4                                   | 5,000                                               | 10.0                                  |
| Serine                              | -                                  | -                                     | 8,600                                                    | 4.8                                   | 6,000                                               | 12.0                                  |
| Methionine                          | -                                  | -                                     | -                                                        | -                                     | 4,000                                               | 8.0                                   |
| Methionine sulphoxide               | 16,800                             | 32.3                                  | 38,300                                                   | 21.2                                  | 9,300                                               | 18.6                                  |
| Homocysteine                        | 5,000                              | 9.6                                   | 88,000                                                   | 48.6                                  | 8,000                                               | 16.0                                  |
| Unknown                             | 12,700                             | 24.4                                  | 16,500                                                   | 9.1                                   | 14,700                                              | 29.4                                  |
| Total Cl <sup>14</sup> incorporated | 52,000                             |                                       | 180,800                                                  |                                       | 50,000                                              |                                       |





TABLE 16

The Effect of homocysteine and glycine on the metabolism of formate-Cl<sup>14</sup> by Radish cotyledons in nitrogen, neutral and basic amino acid fraction

Experimental procedure as in table 15.

| Amino Acids                         | formate-Cl <sup>14</sup> : control |                                       | formate-Cl <sup>14</sup> +<br>4 $\mu$ moles homocysteine |                                       | formate-Cl <sup>14</sup> +<br>4 $\mu$ moles glycine |                                       |
|-------------------------------------|------------------------------------|---------------------------------------|----------------------------------------------------------|---------------------------------------|-----------------------------------------------------|---------------------------------------|
|                                     | Cl <sup>14</sup> (cpm)             | % of Cl <sup>14</sup><br>incorporated | Cl <sup>14</sup> (cpm)                                   | % of Cl <sup>14</sup><br>incorporated | Cl <sup>14</sup> (cpm)                              | % of Cl <sup>14</sup><br>incorporated |
| Glycine                             | 4,500                              | 3.6                                   | 18,000                                                   | 10.7                                  | 57,300                                              | 37.4                                  |
| Serine                              | 10,500                             | 8.4                                   | 15,400                                                   | 9.2                                   | 24,700                                              | 16.1                                  |
| Methionine                          | -                                  | -                                     | 21,400                                                   | 12.7                                  | -                                                   | -                                     |
| Methionine sulphoxide               | 66,500                             | 53.2                                  | 26,700                                                   | 15.9                                  | 36,500                                              | 23.8                                  |
| Homocysteine                        | 13,000                             | 10.4                                  | 31,000                                                   | 18.4                                  | -                                                   | -                                     |
| Unknown                             | 30,500                             | 24.4                                  | 55,500                                                   | 33.0                                  | 34,500                                              | 22.5                                  |
| Total Cl <sup>14</sup> incorporated | 125,000                            |                                       | 168,000                                                  |                                       | 153,000                                             |                                       |



the control tissues showed that glycine, methionine sulphoxide and an unknown compound were the main labelled components. In the presence of homocysteine, however, the bulk of the carbon-14 was recovered in homocysteine and methionine sulphoxide. Additions of glycine resulted in decreases of carbon-14 in glycine and methionine sulphoxide as compared to the control. However, the total carbon-14 entering serine was more than double by addition of glycine.

When the tissues were incubated in nitrogen, incorporation of carbon-14 from formate-C<sup>14</sup> into amino acids fraction was increased by 2.5 times in the control and by 3 times in the presence of glycine. In the control experiment, methionine sulphoxide and an unknown compound accounted for most of the radioactivity. In addition, smaller amounts of carbon-14 were detected in glycine and serine. Addition of homocysteine to the tissues resulted in the production of labelled methionine, methionine sulphoxide and homocysteine. In the presence of glycine, the carbon-14 content of serine was doubled.

It appears therefore that anaerobic conditions are more favorable for the utilization of formate in amino acid biosynthesis. Also additions of glycine and homocysteine stimulated incorporation of formate carbon into serine, methionine and methionine sulphoxide.

#### Pea Leaves

The effects of micromolar amounts of homocysteine and glycine on incorporation of formate into the neutral and basic amino acid fraction in excised pea leaves were studied in light and dark (Table 17, 18). The amino acid fraction was separated



TABLE 17

The Effect of homocysteine and glycine on formate-C14 metabolism in excised Pea leaves\* in light\*\*\*, neutral and basic amino acid fraction

0.25 g slices of leaves incubated at 30° for 1 hr. with 100 µmoles of phosphate buffer (pH 5.6) and 1 µmole of formate containing 2 µc of C14.

| Amino Acids            | formate-C14 : control |                | formate-C14 +<br>8 µmoles homocysteine |                | formate-C14 +<br>16 µmoles glycine |                |
|------------------------|-----------------------|----------------|----------------------------------------|----------------|------------------------------------|----------------|
|                        | % of C14              |                | % of C14                               |                | % of C14                           |                |
|                        | C14 (cpm)             | incorporated** | C14 (cpm)                              | incorporated** | C14 (cpm)                          | incorporated** |
| Glycine                | 4,500                 | 34.7           | -                                      | -              | 4,200                              | 32.3           |
| Alanine                | 2,900                 | 22.4           | 5,000                                  | 5.9            | 1,800                              | 13.8           |
| Serine                 | 2,850                 | 21.9           | 7,500                                  | 8.8            | 3,000                              | 23.1           |
| Methionine             | 100                   | 0.7            | 9,400                                  | 11.1           | -                                  | -              |
| Methionine sulphoxide  | 150                   | 1.1            | 51,600                                 | 60.9           | -                                  | -              |
| Homocysteine           | 2,500                 | 19.2           | 11,200                                 | 13.2           | 4,000                              | 30.7           |
| Total C14 incorporated | 13,000                |                | 84,700                                 |                | 13,000                             |                |

\* excised from 15 day old seedlings

\*\* % of C14 incorporated of the total neutral and basic amino acid fraction

\*\*\* 400 f.c.





TABLE 18

The Effect of homocysteine and glycine on formate-C14 metabolism in excised pea leaves\* in dark, neutral and basic amino acid fraction

Experimental procedure as in table 17.

| Amino Acids            | formate-C14 : control |                | formate-C14 +<br>8 $\mu$ moles homocysteine<br>% of C14 |                | formate-C14 +<br>16 $\mu$ moles glycine<br>% of C14 |                |
|------------------------|-----------------------|----------------|---------------------------------------------------------|----------------|-----------------------------------------------------|----------------|
|                        | C14 (cpm)             | incorporated** | C14 (cpm)                                               | incorporated** | C14 (cpm)                                           | incorporated** |
| Glycine                | 1,400                 | 16.7           | -                                                       | -              | 1,100                                               | 6.7            |
| Serine                 | 2,200                 | 26.2           | 26,000                                                  | 27.7           | 6,600                                               | 40.5           |
| Methionine             | -                     | -              | 4,400                                                   | 4.7            | -                                                   | -              |
| Methionine sulphoxide  | 3,100                 | 36.9           | 48,600                                                  | 51.7           | 5,000                                               | 30.7           |
| Homocysteine           | 1,700                 | 20.2           | 15,000                                                  | 15.9           | 3,000                                               | 18.4           |
| Others                 | -                     | -              | -                                                       | -              | 600                                                 | 3.6            |
| Total C14 incorporated | 8,400                 |                | 94,000                                                  |                | 16,300                                              |                |

\* excised from 15 day old seedlings

\*\* % of C14 incorporated of the total neutral and basic amino acid fraction



using ion exchange and paper chromatography as described earlier.

Incorporation of formate- $C^{14}$  into the neutral and basic amino acid fraction was enhanced more than 6 times in light and 11 times in the dark when homocysteine was added. Additions of glycine doubled the carbon-14 content of this fraction only when the tissues were darkened.

In the illuminated control, most of the carbon-14 was present in glycine, alanine and serine. However, in the presence of homocysteine, 11.1% of the radioactivity was present in methionine and 60.9% in methionine sulphoxide, a common oxidation product of methionine. Clearly, additions of homocysteine enhanced the incorporation of label into methionine in these tissues. Glycine had no significant effect on the incorporation of carbon-14 into amino acids when the tissues were illuminated.

In the darkened tissues, glycine, serine and methionine sulphoxide were chief products in the control. Additions of homocysteine considerably increased the incorporation of carbon-14 into methionine and methionine sulphoxide, and glycine additions greatly stimulated incorporation of formate into serine.

The results of these experiments indicate that glycine and homocysteine have important effects on the extent and nature of formate metabolism in the tissues examined. The results might be interpreted as indicating that formate utilization involves transmethylation reactions in which glycine and homocysteine accept formate carbon to produce serine



and methionine respectively.





### Formic Dehydrogenase Activity in Plant Tissues

In all the plant tissues which were examined for formate- $C^{14}$  metabolism, carbon dioxide was the chief product. The presence of formic dehydrogenase in various plant tissues has been demonstrated by Davison (1949), Mathews and Vennesland (1950), and Mazelis (1960). However, little is known about the changes in this enzyme's activity during germination of seeds or of its intracellular localization. An attempt has therefore been made to follow possible changes in formic dehydrogenase activity during germination of castor bean and pea seeds and also to determine if the enzyme is intracellularly localized in these tissues.

#### Germinating Pea Cotyledons

Changes in the formic dehydrogenase activity of germinating pea cotyledons were followed over a 5-day period (Table 19). In the mitochondrial fraction, the enzyme activity rose to a maximum in four-day old cotyledons. Additions of NAD only slightly increased the formic dehydrogenase activity of the mitochondrial preparations. The formic dehydrogenase obtained from the high speed supernatant was more active than that of the mitochondrial fraction, both on the basis of unit fresh weight and protein nitrogen. Again additions of NAD only gave slight enhancement of  $CO_2$  production. The results are therefore in agreement with earlier reports (Davies 1955) that formic dehydrogenase is present in germinating pea cotyledons. The present studies also indicate that this enzyme is present in the mitochondrial fraction and in the high speed supernatant fraction throughout the early stages of



TABLE 19

The Intracellular distribution of formic dehydrogenase activity in germinating Pea cotyledons

Each reaction flask contained: 1 ml enzyme in sucrose-phosphate buffer (pH 5.6); 5  $\mu$ moles of formate containing 0.05  $\mu$ c of Cl4 incubated for 1 hr. at 300. Mitochondrial and high speed supernatant fractions were prepared as described in Methods Section. Additions of NAD were made as indicated.

| Age of<br>Cotyledons | Enzyme activity as Cl <sup>4</sup> O <sub>2</sub> (cpm)/g.f. wt/hr. |                            |                        |                            | Enzyme activity as Cl <sup>4</sup> O <sub>2</sub> (cpm)/mg protein N/hr. |                            |                        |                            |
|----------------------|---------------------------------------------------------------------|----------------------------|------------------------|----------------------------|--------------------------------------------------------------------------|----------------------------|------------------------|----------------------------|
|                      | Mitochondrial fraction                                              |                            | High speed supernatant |                            | Mitochondrial fraction                                                   |                            | High speed supernatant |                            |
|                      | Enzyme<br>Control                                                   | Enzyme +<br>50 $\mu$ g NAD | Enzyme<br>Control      | Enzyme +<br>50 $\mu$ g NAD | Enzyme<br>Control                                                        | Enzyme +<br>50 $\mu$ g NAD | Enzyme<br>Control      | Enzyme +<br>50 $\mu$ g NAD |
| Days                 |                                                                     |                            |                        |                            |                                                                          |                            |                        |                            |
| 1                    | 157                                                                 | 210                        | 5,250                  | 5,340                      | 2,450                                                                    | 3,000                      | 6,250                  | 6,350                      |
| 2                    | 325                                                                 | 290                        | 4,150                  | 9,800                      | 2,700                                                                    | 2,400                      | 3,800                  | 8,900                      |
| 3                    | 322                                                                 | 400                        | 14,650                 | 16,700                     | 1,800                                                                    | 2,200                      | 5,060                  | 5,800                      |
| 4                    | 1,000                                                               | 980                        | 13,100                 | 17,000                     | 7,100                                                                    | 5,500                      | 6,680                  | 8,070                      |
| 5                    | 296                                                                 | 300                        | 17,950                 | 16,300                     | 3,020                                                                    | 3,060                      | 9,160                  | 7,300                      |



germination.

#### Castor Bean Endosperm

In castor bean endosperm, the activity of formic dehydrogenase fluctuated over the 5-day germination period but was maximal in the youngest tissues (Table 20, 21). Additions of NAD enhanced  $\text{CO}_2$  production slightly in the mitochondrial fraction, but more than 3 times in the supernatant fraction. The production of  $\text{CO}_2$  was also stimulated by NADP but to a lesser extent as compared to NAD.

Determinations of protein nitrogen, using aliquots of the enzyme solutions, indicated that the formic dehydrogenase in the mitochondrial fraction had a higher specific enzyme activity. However, of the total formic dehydrogenase activity extracted from the tissues, the bulk was present in the high speed supernatant fraction.

#### Excised Corn Roots

The root tips (5 mm) of the 3-day old corn seedlings showed considerable formic dehydrogenase activity (Table 22). The formic dehydrogenase activity was enhanced 19% by additions of NAD and by 6% with NADP. Additions of inhibitors such as azide, iodoacetate and cyanide inhibited the enzyme activity. Maximum inhibition was observed with cyanide.







TABLE 20

The Intracellular distribution of formic dehydrogenase activity in germinating Castor bean endosperm

Each reaction flask contained: 1 ml enzyme in sucrose-phosphate buffer (pH 5.6), 5  $\mu$ moles of formate containing 0.05  $\mu$ c of Cl4 in a total volume of 1.2 ml, incubated for 1 hr. at 30°. Mitochondrial and high speed supernatant fractions were prepared as described in Methods Section. Additions of NAD or NADP were made as indicated. Enzyme activity expressed as Cl4O2 (cpm)/g.f. wt/1 hr.

| Age of Castor Bean<br>Endosperm<br>Days | Mitochondrial Fraction |                            | High Speed Supernatant Fraction |                            |
|-----------------------------------------|------------------------|----------------------------|---------------------------------|----------------------------|
|                                         | Enzyme Control         | Enzyme +<br>50 $\mu$ g NAD | Enzyme Control                  | Enzyme +<br>50 $\mu$ g NAD |
|                                         |                        | 50 $\mu$ g NADP            |                                 | 50 $\mu$ g NADP            |
| 1                                       | 240                    | 280                        | 960                             | 3,070                      |
| 2                                       | 135                    | 150                        | 420                             | 1,330                      |
| 3                                       | 96                     | 100                        | 310                             | 990                        |
| 4                                       | 300                    | 300                        | 610                             | 2,160                      |
| 5                                       | 290                    | 290                        | 1,420                           | 3,050                      |
| 8                                       | 210                    | 250                        | 600                             | 900                        |
|                                         |                        |                            |                                 | -                          |



TABLE 21

The Intracellular distribution of formic dehydrogenase activity in germinating Castor bean endosperm

Each reaction flask contained: 1 ml enzyme in sucrose-phosphate buffer (pH 5.6), 5  $\mu$ moles of formate containing 0.05  $\mu$ c of Cl4 in a total volume of 1.2 ml, incubated for 1 hr. at 30°. Mitochondrial and high speed supernatant fractions were prepared as described in Methods Section. Additions of NAD or NADP were made as indicated. Enzyme activity expressed as Cl4O<sub>2</sub> (cpm)/mg protein N/hr.

| Age of Castor Bean<br>Endosperm<br>Days | Mitochondrial Fraction |                |                 | High Speed Supernatant Fraction |                |                 |
|-----------------------------------------|------------------------|----------------|-----------------|---------------------------------|----------------|-----------------|
|                                         | Enzyme Control         | Enzyme +       |                 | Enzyme Control                  | Enzyme +       |                 |
|                                         |                        | 50 $\mu$ g NAD | 50 $\mu$ g NADP |                                 | 50 $\mu$ g NAD | 50 $\mu$ g NADP |
| 1                                       | 11,530                 | 13,500         | 9,830           | 1,280                           | 4,060          | 2,400           |
| 2                                       | 2,700                  | 3,050          | 4,100           | 400                             | 1,250          | 890             |
| 3                                       | 2,550                  | 2,720          | 2,250           | 200                             | 630            | 430             |
| 4                                       | 6,140                  | 6,140          | -               | 430                             | 1,500          | 700             |
| 5                                       | 6,300                  | 6,300          | 6,200           | 1,100                           | 2,400          | 1,800           |
| 8                                       | 5,230                  | 7,150          | -               | 2,260                           | 4,000          | -               |



TABLE 22

The Formic dehydrogenase activity in mitochondria of excised Corn roots\*

Each reaction flask contained: 1 ml enzyme in sucrose-tris buffer (pH 7.4); 5  $\mu$ moles of formate containing 1  $\mu$ c of  $\text{Cl}^{14}$  incubated for 1 hr. at 30°. Mitochondrial fraction was prepared as described in Methods Section. Additions of NAD, NADP and inhibitors were made as indicated.

| <u>Treatment</u>                                              | <u>Formic dehydrogenase activity<br/>as <math>\text{Cl}^{14}\text{O}_2</math> (cpm)/g.f. wt/hr.</u> | <u>Enzyme activity<br/>(%) over control</u> |
|---------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|---------------------------------------------|
| Enzyme - control                                              | 8,000                                                                                               | 100                                         |
| Enzyme + 50 $\mu$ g NAD                                       | 9,500                                                                                               | 118.8                                       |
| Enzyme + 50 $\mu$ g NADP                                      | 8,500                                                                                               | 106.2                                       |
| Enzyme + 50 $\mu$ g NAD + $10^{-3}\text{M}$ azide             | 6,700                                                                                               | 83.7                                        |
| Enzyme + 50 $\mu$ g NAD + $10^{-3}\text{M}$ iodoacetate       | 5,300                                                                                               | 66.2                                        |
| Enzyme + 50 $\mu$ g NAD + $10^{-3}\text{M}$ potassium cyanide | 3,000                                                                                               | 37.2                                        |

\* excised from 3 day old seedlings





## The Metabolism of Glycine-C<sup>14</sup> by Plant Tissues

Formate-C<sup>14</sup> feeding experiments using a variety of plant tissues have indicated that this compound may be involved in the biosynthesis of amino acids such as serine and methionine (see earlier section). Furthermore, additions of glycine and homocysteine altered the extent and products of formate metabolism in a way that suggests the possible involvement of transmethylation reactions in formate utilization.

Also, if the biosynthesis of serine in these tissues involves a transmethylation reaction in which glycine participates, experiments with glycine-C<sup>14</sup> should result in the incorporation of glycine-carbon into carbons 1 and 2 of serine. Furthermore, it is possible that the 2 position of glycine might be converted to a 'formyl' radical or formate (as mentioned earlier) and so enter the 3 position of serine.

In order to examine these possibilities, experiments with glycine-C<sup>14</sup> solutions were carried out, using tissues known to produce serine from formate-C<sup>14</sup>.

## The Metabolism of Glycine by Castor Bean Endosperm Slices

During the six hour experimental period, the castor bean endosperm slices metabolized approximately 46% of the glycine-C<sup>14</sup> supplied (Table 23). This utilization resulted in considerable amounts of radioactivity being incorporated into all the fractions isolated. The major products of glycine metabolism were serine and carbon dioxide which accounted for 33% and 31% respectively of the carbon-14 incorporated. In addition, radioactivity was present in the acidic amino acid and organic acid fractions. Although the percentage of



TABLE 23

The Utilization of glycine-C<sup>14</sup> by Castor bean endosperms

1 g endosperm slices of 5-day old seedlings incubated for 6 hr. at 30° in Warburg flasks containing:- 300 µmoles tris buffer at pH 7.2, 0.1 ml glycine-C<sup>14</sup> (2 µc/µmole), 326,000 counts/min. of C<sup>14</sup>, total volume 1.6 ml. Tissues were killed and extracted as described in the text.

| <u>Fraction</u>                       | <u>Counts/min.</u> | <u>% of C<sup>14</sup> incorporated</u> |
|---------------------------------------|--------------------|-----------------------------------------|
| Lipids                                | 2,400              | 1                                       |
| Amino Acids                           |                    |                                         |
| Neutral & Basic<br>amino acids        |                    |                                         |
| serine                                | 49,700             | 33                                      |
| Acidic amino acids                    | 23,300             | 15                                      |
| Sugars                                | 3,900              | 3                                       |
| Organic Acids                         |                    |                                         |
| Glyoxylate                            | 6,760              | 4                                       |
| Glycollate                            | 2,050              | 1                                       |
| Succinate                             | 1,150              | 0.7                                     |
| Malate                                | 1,070              | 0.7                                     |
| Citrate                               | 460                | 0.3                                     |
| Others                                | 810                | 0.5                                     |
| Carbon Dioxide                        | 47,600             | 31                                      |
| Residue                               | 12,500             | 8                                       |
| Total C <sup>14</sup> incorporated    | 150,700            |                                         |
| % of glycine-C <sup>14</sup> utilized | 46                 |                                         |



carbon-14 incorporated into the organic acids of castor bean endosperm was less than occurred into the acidic amino acids, the label was mainly present in glyoxylate and glycollate. Smaller amounts of carbon-14 were detected in malate, succinate, in lipid material and in the sugars.

#### The Metabolism of Glycine by Carrot Tissues

Following a six hour incubation with glycine-C<sup>14</sup>, the carrot tissues metabolized 99% of the supplied carbon-14 (Table 24). The largest percentage of carbon-14 incorporated, was found in the insoluble material. As in castor bean slices, considerable amounts of radioactivity were present in the carbon dioxide evolved and serine was the major radioactive component of the soluble fractions isolated.

Radioactivity of the organic acid fraction (Table 25) was due mainly to large amounts of labelling in glyoxylate as occurred in the castor bean endosperm. In addition, malate, succinate, citrate and the pyruvate fraction contained carbon-14 derived from glycine.

In experiments where the time of incubation with glycine-2-C<sup>14</sup> solutions was reduced (Table 26), the distribution of carbon-14 in the various fractions was considerably altered. After 5 minutes of glycine metabolism, the organic acids were the main products. In contrast, only small amounts of glycine-carbon were converted to carbon dioxide. As the time of incubation with the glycine-C<sup>14</sup> solutions was increased to 90 minutes, the percentage of total carbon-14 incorporated into organic acids decreased. This was accompanied by increases in the percentage of carbon-14 found in the in-







TABLE 24

The Utilization of glycine-Cl<sup>14</sup> by Carrot tissues

1 g cortical tissues of Carrot roots incubated for 6 hr. at 30° in Warburg flasks containing:-  
 200 µmoles tris buffer at pH 7.2, 0.1 ml glycine-Cl<sup>14</sup> (2 µc/µmole), 427,000 counts/min. of Cl<sup>14</sup>,  
 10 µmoles sodium formate (0.1 ml) or 0.1 ml distilled water as indicated. Total volume 1.2 ml.  
 Tissue killed and extracted as described in text.

| Fraction                               | glycine-Cl <sup>14</sup> |                                    | glycine-Cl <sup>14</sup> + formate |                                    |
|----------------------------------------|--------------------------|------------------------------------|------------------------------------|------------------------------------|
|                                        | Counts/min.              | % of Cl <sup>14</sup> incorporated | Counts/min.                        | % of Cl <sup>14</sup> incorporated |
| Lipids                                 | 6,300                    | 1.5                                | 5,600                              | 1.5                                |
| Amino Acids                            |                          |                                    |                                    |                                    |
| Neutral & Basic amino acids            |                          |                                    |                                    |                                    |
| serine                                 | 90,740                   | 21                                 | 2,600                              | 0.6                                |
| Acidic amino acids                     | 25,000                   | 6                                  | 22,800                             | 6                                  |
| Sugars                                 | 1,900                    | 0.4                                | 3,600                              | 0.9                                |
| Organic Acids                          | 27,000                   | 6                                  | 49,400                             | 13                                 |
| Carbon Dioxide                         | 117,750                  | 27                                 | 116,200                            | 31                                 |
| Residue                                | 155,250                  | 36                                 | 178,000                            | 47                                 |
| Total Cl <sup>14</sup> incorporated    | 426,540                  |                                    | 378,200                            |                                    |
| % of glycine-Cl <sup>14</sup> utilized | 99                       |                                    | 88                                 |                                    |



TABLE 25

Distribution of Cl4 from glycine-Cl4 in the organic acids of Carrot tissues

1 g cortical tissues of carrot roots incubated for 6 hr. at 30° in Warburg flasks containing:- 200 µmoles tris buffer at pH 7.2, 0.1 ml glycine-Cl4 (2 µc/µmole) 427,000 counts/min. of Cl4. Additions of 10 µmoles sodium formate, 100 µmoles sodium iodoacetate and sodium malonate were made as indicated. Total volume 1.2 ml.

| Organic Acid                       | glycine-Cl4 |                           | glycine-Cl4 + formate |                           | glycine-Cl4 + iodoacetate |                           | glycine-Cl4 + malonate |                           |
|------------------------------------|-------------|---------------------------|-----------------------|---------------------------|---------------------------|---------------------------|------------------------|---------------------------|
|                                    | Counts/min. | % of Cl4 in organic acids | Counts/min.           | % of Cl4 in organic acids | Counts/min.               | % of Cl4 in organic acids | Counts/min.            | % of Cl4 in organic acids |
| Glyoxylate                         | 10,200      | 38                        | 11,000                | 22                        | 7,300                     | 33                        | 2,900                  | 54                        |
| Glycollate                         | not active  | -                         | 3,100                 | 6                         | not active                | -                         | 1,700                  | 32                        |
| Succinate                          | 2,500       | 9                         | 4,500                 | 9                         | 2,200                     | 10                        | 700                    | 13                        |
| Malate                             | 5,500       | 20                        | 21,700                | 43                        | 1,600                     | 7                         | not active             | -                         |
| Citrate                            | 2,300       | 8                         | 2,000                 | 4                         | not active                | -                         | not active             | -                         |
| Pyruvate fraction                  | 2,500       | 9                         | 4,200                 | 8                         | 8,200                     | 37                        | not active             | -                         |
| Others                             | 4,000       | 15                        | 2,900                 | 6                         | 2,700                     | 12                        | not active             | -                         |
| Total Cl4 of organic acid fraction | 27,000      |                           | 49,400                |                           | 22,000                    |                           | 5,300                  |                           |



TABLE 26

Sequence of incorporation of  $\text{Cl}^{14}$  into products of glycine- $\text{Cl}^{14}$  metabolism in Carrot tissue slices

1 g cortical tissues of carrot roots incubated at  $30^{\circ}$  for time periods as indicated. Slices incubated in 200  $\mu\text{moles}$  tris buffer (pH 7.2) and 0.1 ml of glycine- $\text{Cl}^{14}$  (2  $\mu\text{c}/\mu\text{mole}$ ), 427,000 counts/min. of  $\text{Cl}^{14}$ . Total volume 1.1 ml.

| Fraction                                | Time of incubation |                                    |             |                                    |             |                                    |
|-----------------------------------------|--------------------|------------------------------------|-------------|------------------------------------|-------------|------------------------------------|
|                                         | 5 minutes          |                                    | 30 minutes  |                                    | 90 minutes  |                                    |
|                                         | Counts/min.        | % of $\text{Cl}^{14}$ incorporated | Counts/min. | % of $\text{Cl}^{14}$ incorporated | Counts/min. | % of $\text{Cl}^{14}$ incorporated |
| Lipids                                  | 200                | 7                                  | 500         | 6                                  | 600         | 3                                  |
| Sugars                                  | 300                | 10                                 | 300         | 4                                  | 900         | 5                                  |
| Organic Acids                           | 1,800              | 63                                 | 4,000       | 49                                 | 6,300       | 32                                 |
| Carbon Dioxide                          | 60                 | 3                                  | 500         | 6                                  | 1,200       | 6                                  |
| Residue                                 | 500                | 17                                 | 2,900       | 35                                 | 10,500      | 54                                 |
| Total $\text{Cl}^{14}$ incorporated     | 2,860              |                                    | 8,200       |                                    | 19,500      |                                    |
| % of glycine- $\text{Cl}^{14}$ utilized | 0.67               |                                    | 1.9         |                                    | 4.5         |                                    |





soluble residue and in the carbon dioxide evolved during the experiments.

#### The Effect of Formate on Glycine Metabolism in Carrot Tissue

The effect of adding formate to carrot tissues metabolizing glycine is shown in Table 24. In the presence of the 10  $\mu$ moles of formate, the amount of carbon-14 entering the fractions isolated was reduced to 88% of the carbon-14 supplied. Furthermore, the soluble amino acids contained only small amounts of serine-C<sup>14</sup> when formate was supplied. Additions of formate were, however, clearly associated with increased amounts of carbon-14 being incorporated into the insoluble residue and the organic acid fraction. Hydrolysis of this insoluble material indicated that the major radioactive component was serine. Hence, additions of formate, although not leading to large amounts of serine-C<sup>14</sup> in the free amino acids, was associated with an increase in the incorporation of glycine-C<sup>14</sup> into protein serine.

The effect of additions of formate on the distribution of radioactivity in the organic acid fraction is shown in Table 25. When formate was added to the slices, greater amounts of glycine-C<sup>14</sup> were converted to malic acid. The radioactivity present in glyoxylate was not appreciably altered by this treatment, but glycollate was now detected and the radioactivity of succinate almost doubled.

#### The Effects of Iodoacetate and Malonate on the Metabolism of Glycine-C<sup>14</sup> by Carrot Tissues

In studies on the effects of inhibitors on the metabolism of glycine-C<sup>14</sup>, carrot tissue slices were incubated with



TABLE 27

The effect of iodoacetate and malonate on the metabolism of glycine-C14 by Carrot tissue slices  
 1 g cortical tissues of carrot root incubated for 6 hr. at 30° as indicated in Table 3.

|                                | glycine-C14        |                                  | glycine-C14 +<br>iodoacetate |                                  | glycine-C14 +<br>malonate |                                  |
|--------------------------------|--------------------|----------------------------------|------------------------------|----------------------------------|---------------------------|----------------------------------|
|                                | <u>Counts/min.</u> | <u>% of C14<br/>incorporated</u> | <u>Counts/min.</u>           | <u>% of C14<br/>incorporated</u> | <u>Counts/min.</u>        | <u>% of C14<br/>incorporated</u> |
| Lipids                         | 6,300              | 1.5                              | 3,200                        | 2                                | 300                       | 4                                |
| Amino Acids                    |                    |                                  |                              |                                  |                           |                                  |
| Neutral & Basic<br>amino acids |                    |                                  |                              |                                  |                           |                                  |
| serine                         | 90,740             | 21                               | not active                   |                                  | not active                |                                  |
| Acidic amino acids             | 25,000             | 6                                | not active                   |                                  | not active                |                                  |
| Sugars                         | 1,900              | 0.4                              | 1,200                        | 0.7                              | 500                       | 6                                |
| Organic Acids                  | 27,000             | 6                                | 22,000                       | 13                               | 5,300                     | 64                               |
| Carbon Dioxide                 | 117,750            | 27                               | 76,900                       | 47                               | 2,100                     | 25                               |
| Residue                        | 155,250            | 36                               | 60,400                       | 36                               | not active                |                                  |
| Total C14<br>incorporated      | 426,540            |                                  | 163,700                      |                                  | 8,200                     |                                  |
| % of glycine-C14<br>utilized   | 99                 |                                  | 38                           |                                  | 1.9                       |                                  |



the inhibitor for 30 minutes prior to additions of the glycine- $C^{14}$ . As is evident from Table 27, the presence of 100  $\mu$ moles of iodoacetate and 100  $\mu$ moles of malonate, greatly reduced the amounts of glycine- $C^{14}$  metabolized. In the control slices, 99% of the glycine was metabolized, this was decreased to 38% and 1.9% in the presence of iodoacetate and malonate respectively.

Inhibition by iodoacetate and malonate affected the radioactivity of all the fractions isolated. Furthermore, in the presence of 100  $\mu$ moles of iodoacetate, the distribution of carbon-14 in the various fractions was strikingly altered from that observed in the control slices. In the control tissues, 27% of the incorporated glycine- $C^{14}$  was evolved as carbon dioxide; in the iodoacetate treated slices, this figure was increased to 47%. Similarly, the percentage of carbon-14 incorporated into the organic acids was increased from 6% in the controls to 13% in the iodoacetate treated tissues. As is shown in Table 25, iodoacetate treatment resulted in large decreases in the activities recovered in glyoxylate and malate. In contrast, increases were observed in the carbon-14 content of the pyruvate fraction in the presence of iodoacetate.

Malonate, besides dramatically reducing the amounts of glycine- $C^{14}$  metabolized, also greatly altered the distribution of carbon-14 in the fractions isolated. Radioactivity was not detected in the insoluble residue in the malonate treated tissues but comprised 36% of carbon-14 incorporated in the control (Table 27). Malonate treatment also altered the percentage of carbon-14 in the organic acid fraction from 6% in







the control to 64% in the inhibited slices. The effect of malonate on the organic acids was further evident when this fraction was further fractionated (Table 25). Following malonate treatment, carbon-14 was present only in glyoxylate, glycollate and succinate. Radioactivity was not detected in malate, citrate or in the pyruvate fraction when malonate was present.

#### Intramolecular Distribution of Carbon-14 in Glycine and Serine

Table 28 shows the percentage distribution of carbon-14 in the glycine solutions fed to the tissues and the distribution found in samples of glycine extracted from the tissues after 6 hours metabolism of glycine-C<sup>14</sup>. The glycine fed to the tissues was found to have 66% of carbon-14 in the 2 position and 34% in the 1 position. Following incubation with the tissues, this intramolecular distribution of carbon-14 was greatly altered. In both cases, greater percentages of carbon-14 were found in the 2 position of the glycine molecule.

Degradations of serine-C<sup>14</sup> produced by the carrot tissues metabolizing glycine-C<sup>14</sup> (Table 28), indicated that 50% of the carbon-14 content of the molecule was located in the 3 position. In contrast, the 3 position of serine produced by castor bean endosperm contained only 7% of the carbon-14 content of the serine molecule.

#### Germinating Sunflower Cotyledons

The metabolism of glycine-2-C<sup>14</sup> by sunflower cotyledons was examined over a 6-day period of germination. As is indicated in Table 29a, 2  $\mu$ c of carbon-14 containing approximately  $6 \times 10^5$  cpm in glycine-2-C<sup>14</sup> were supplied to samples of



TABLE 28

Intramolecular distribution of  $C^{14}$  in glycine and serine following feeding with glycine- $C^{14}$

Samples (10,000 counts/min.) of labelled glycine used in the feeding experiments or recovered from tissues after feeding for 6 hr. at  $30^{\circ}$  (see Methods) were mixed with 10  $\mu$ moles carrier glycine and decarboxylated using ninhydrin. Activity in 2 position is calculated by difference between counts/min. of sample and counts/min. recovered after ninhydrin treatment. Samples of serine- $C^{14}$  recovered from tissues after 6 hr. glycine- $C^{14}$  metabolism were degraded using periodate (see Methods). Expressed as percentage of  $C^{14}$  recovered from products of degradation procedures.

| Amino Acid | Carbon Number | Original Feeding<br>Soln. | Extracted From |             |
|------------|---------------|---------------------------|----------------|-------------|
|            |               |                           | Carrot         | Castor Bean |
| Glycine    | 1             | 34                        | 24             | 26          |
|            | 2             | 66                        | 76             | 74          |
| Serine     | 1             | -                         | 17.8           | 21.1        |
|            | 2             | -                         | 28.2           | 71.9        |
|            | 3             | -                         | 54.0           | 7.0         |



TABLE 29a

The Metabolism of Glycine-2-C<sup>14</sup> by germinating Sunflower cotyledons

0.5 g slices of cotyledons incubated at 30° for 6 hr. in darkness with 100 μmoles of phosphate buffer (pH 5.6) and 1 μmole of glycine-2-C<sup>14</sup> containing 2 μc of C<sup>14</sup>. Radioactivity expressed as cpm.

| Fractions                          | Age of Cotyledons (days) |            |         |         |   |         |
|------------------------------------|--------------------------|------------|---------|---------|---|---------|
|                                    | 1                        | 2          | 3       | 5       | 6 |         |
| Carbon Dioxide                     | 22,000                   | 16,000     | 34,000  | 45,000  |   | 56,000  |
| Lipids                             | 1,800                    | 3,000      | 3,000   | 5,000   |   | 6,000   |
| Sugars                             | 3,600                    | 5,000      | 5,000   | 7,500   |   | 20,000  |
| Organic Acids                      | 12,000                   | 12,000     | 12,000  | 18,700  |   | 47,000  |
| Amino Acids                        |                          |            |         |         |   |         |
| Serine                             | 172,000                  | 251,000    | 210,000 | 140,000 |   | 129,000 |
| Glutamic & Aspartic acid           | not active               | not active | 14,000  | 51,000  |   | 49,000  |
| Others                             | not active               | not active | 9,000   | 21,000  |   | 26,000  |
| Residue                            | 52,000                   | 137,000    | 137,000 | 153,000 |   | 200,000 |
| Total C <sup>14</sup> incorporated | 263,400                  | 424,000    | 424,400 | 441,200 |   | 533,000 |





cotyledon slices. In the earliest stages of germination, only 43.9% of the glycine supplied was metabolized in six hours. However, with further germination, greater amounts of the supplied glycine were metabolized. The 6-day old tissues metabolized over 80% of the glycine-C<sup>14</sup> fed.

At all stages of germination, serine-C<sup>14</sup> was the chief product of glycine-2-C<sup>14</sup> metabolism. However, the percentage of carbon-14 entering serine was greatest in the youngest tissues (Table 29b), decreasing in the 6-day old cotyledons. During the germination period, there was, however, an increase in the percentage of carbon-14 entering the insoluble residue. Although the sugars and organic acids fractions were labelled, the percentage of carbon-14 in these fractions was comparatively small. As in the carrot and castor bean tissues, the main organic acids produced from glycine by the sunflower cotyledons were glyoxylate and glycollate. The degradation of serine produced by 6-day old cotyledons showed no carbon-14 in the 1 position. The 2 and 3 positions of serine accounted for 84% and 16% of the activity of the molecule. In this regard, results are more similar to castor bean endosperm than carrot tissue.

The results of the present studies with glycine-C<sup>14</sup> metabolism have clearly indicated that the carbon 1 and 2 of glycine can become the corresponding carbons in serine. Furthermore, in the experiments with sunflower cotyledons, labelling of 3 position of serine indicates that this carbon can arise from the 2 position of glycine. Labelling of glyoxylate and glycollate might indicate the existence of a



TABLE 29b

The Metabolism of glycine-2-C14 by germinating Sunflower cotyledons

Experimental procedure as in table 29a. Results expressed as percentage of total C14 incorporated.

| <u>Fractions</u>         | <u>Age of Cotyledons (days)</u> |            |          |          |          |
|--------------------------|---------------------------------|------------|----------|----------|----------|
|                          | <u>1</u>                        | <u>2</u>   | <u>3</u> | <u>5</u> | <u>6</u> |
| Carbon Dioxide           | 8.3                             | 3.7        | 8.0      | 10.2     | 10.5     |
| Lipids                   | 0.7                             | 0.7        | 0.7      | 1.1      | 1.1      |
| Sugars                   | 1.3                             | 1.2        | 1.2      | 1.7      | 3.8      |
| Organic Acids            | 4.5                             | 2.8        | 2.8      | 4.2      | 8.8      |
| Amino Acids              |                                 |            |          |          |          |
| Serine                   | 65.3                            | 59.2       | 49.5     | 31.8     | 24.2     |
| Glutamic & Aspartic acid | not active                      | not active | 3.3      | 11.6     | 9.2      |
| Others                   | not active                      | not active | 2.1      | 4.8      | 5.0      |
| Residue                  | 19.7                            | 32.1       | 32.1     | 34.6     | 37.2     |



transaminase system in these tissues. In addition, changes in the distribution of carbon-14 in 1 and 2 positions of glycine during the experimental period suggests that this compound is extensively broken down and resynthesized in vivo.





### Glyoxylate Metabolism by Plant Tissues

In studies on glycine- $C^{14}$  metabolism using carrot storage tissues, castor bean endosperm and sunflower cotyledons, glyoxylate contained the greatest amount of carbon-14 present in the organic acids produced during the six hour experimental periods. It is, therefore, possible that glycine might be converted to glyoxylate by a trans-aminase system present in these tissues. Glyoxylate, arising from glycine, might then be utilized by the tissues to give rise to a variety of products. Glyoxylate is also presumed to be produced from glycollate during the photosynthetic fixation of carbon dioxide (Rabson, Tolbert and Kearney 1962). In germinating fatty seeds, glyoxylate is readily produced from isocitrate by the isocitritase reaction (Carpenter and Beevers 1959).

As glyoxylate was readily produced from glycine- $C^{14}$  in the feeding experiments, and as this compound yielded formate (see earlier section), it is possible that glyoxylate may be an important precursor of glycine and serine in these tissues.

If this assumption is correct, the tissues should be able to convert glyoxylate to glycine and synthesize all the carbons of serine from this keto acid.

### Carrot Storage Tissue

In a six hour experimental period, glyoxylate-1,2- $C^{14}$  was converted mainly into carbon dioxide, the insoluble residue and the organic acid fraction (Table 30). Glycine and serine accounted for 14.2% of the total carbon-14 incorporated. Analysis of the organic acid fraction (Table 35) showed that



TABLE 30

The Effect of formate on the metabolism of glyoxylate-1,2-Cl4 by Carrot tissue

0.5 g of slices of storage tissue incubated at 30° for 6 hr. in darkness with 100  $\mu$ moles of phosphate buffer (pH 5.6) and 1  $\mu$ mole of glyoxylate containing 1  $\mu$ c of Cl4.

| Fraction                 | Glyoxylate-1,2-Cl4 |                       | Glyoxylate-1,2-Cl4 +<br>10 $\mu$ moles formate |                       |
|--------------------------|--------------------|-----------------------|------------------------------------------------|-----------------------|
|                          | Cl4 (cpm)          | % of incorporated Cl4 | Cl4 (cpm)                                      | % of incorporated Cl4 |
| Carbon Dioxide           | 104,300            | 31.2                  | 95,000                                         | 29.6                  |
| Lipids                   | 4,500              | 1.3                   | 2,600                                          | 0.8                   |
| Sugars                   | 3,200              | 1.0                   | 2,000                                          | 0.6                   |
| Organic Acids            | 84,500             | 25.3                  | 90,000                                         | 28.0                  |
| Amino Acids              |                    |                       |                                                |                       |
| Glycine                  | 26,700             | 8.0                   | 24,000                                         | 7.4                   |
| Serine                   | 20,600             | 6.2                   | 22,000                                         | 7.0                   |
| Glutamic & Aspartic acid | 8,000              | 2.4                   | 9,400                                          | 3.0                   |
| Residue                  | 82,700             | 24.7                  | 76,000                                         | 23.7                  |
| Total Cl4 incorporated   | 334,000            |                       | 321,000                                        |                       |



it consisted mainly of malate, succinate and oxalate. The carbon-14 content of malate accounted for 6% of the total carbon-14 incorporated and 23.9% of the carbon-14 present in the organic acid fraction. Degradations of the serine-C<sup>14</sup>, produced during glyoxylate-C<sup>14</sup> feeding (Table 36), indicated that glyoxylate carbon had been incorporated into all three carbons of the serine molecule. Carbons 1 and 2 contained the greatest percentage of carbon-14, the 3 position containing only 4.7% of the total carbon-14 content.

A time course study of glyoxylate-1,2-C<sup>14</sup> metabolism by carrot storage tissues showed that within 2 minutes, glycollate and glycine were the major products (Table 31). Carbon dioxide, although accounting for 31.2% of the total carbon-14 incorporated in six hours, had only 0.8% of the total carbon-14 incorporated in 2 minutes. Glycine was the first amino acid to become labelled, although radioactivity was detected in serine after 15 minutes of glyoxylate feeding. Labelling was found in malate and succinate, after 30 minutes, together with an unknown organic acid eluting with the glyceric acid fraction which was radioactive within 5 minutes.

#### The Effect of Formate on Glyoxylate-1,2-C<sup>14</sup> Metabolism in Carrot Tissues

From studies with carrot tissues, it was suggested earlier that glyoxylate might be decarboxylated to yield carbon dioxide and formate. Due to the presence of formic dehydrogenase, formate might be oxidized to carbon dioxide. Therefore, if a large unlabelled pool of formate is added to tissues metabolizing glyoxylate-C<sup>14</sup>, it should suppress the







TABLE 31

The Time course distribution of C14 among the various fractions obtained from Carrot storage tissue slices incubated with glyoxylate-1,2-C14

0.5 g of carrot storage tissue incubated at 30° in darkness with 100 μmoles of phosphate buffer (pH 5.6 and 1 μmole of glyoxylate containing 2 μc of C14.

| Fractions                | Time of incubation in minutes |                  |            |                  |            |                  |
|--------------------------|-------------------------------|------------------|------------|------------------|------------|------------------|
|                          | 2                             |                  | 5          |                  | 15         |                  |
|                          | C14 (cpm)                     | % of incorp. C14 | C14 (cpm)  | % of incorp. C14 | C14 (cpm)  | % of incorp. C14 |
| Carbon Dioxide           | 900                           | 0.8              | 900        | 0.7              | 900        | 0.6              |
| Lipids                   | 3,100                         | 2.7              | 2,300      | 1.7              | 2,800      | 1.9              |
| Sugars                   | not active                    | -                | 1,000      | 0.7              | 1,000      | 0.7              |
| Organic Acids            |                               |                  |            |                  |            |                  |
| Glycollate               | 76,000                        | 67.9             | 95,000     | 69.8             | 95,000     | 63.1             |
| Succinate                | not active                    | -                | not active | -                | not active | -                |
| Malate                   | not active                    | -                | not active | -                | not active | -                |
| Others                   | not active                    | -                | 5,000      | 3.7              | 5,000      | 3.4              |
| Amino Acids              |                               |                  |            |                  |            |                  |
| Glycine                  | 22,400                        | 20.0             | 21,700     | 16.0             | 29,000     | 19.5             |
| Serine                   | not active                    | -                | not active | -                | 2,000      | 1.4              |
| Glutamic & Aspartic acid | 2,600                         | 2.3              | 2,600      | 1.9              | 5,500      | 3.7              |
| Residue                  | 7,000                         | 6.2              | 7,500      | 5.5              | 8,300      | 5.6              |
|                          |                               |                  |            |                  | 10,000     | 5.6              |



production of labelled carbon dioxide. In order to test this, glyoxylate-1,2- $C^{14}$  was fed to carrot tissues in the presence of 10  $\mu$ moles of non-radioactive formate. The results are shown in Table 30. Additions of formate resulted in a marked reduction in the amount of carbon-14 entering the respired carbon dioxide. Furthermore, the specific activity of the carbon dioxide was reduced in the presence of formate (the specific activity of carbonate collected was 10,360 cpm/mg  $BaCO_3$  as compared to 13,900 cpm/mg  $BaCO_3$  in the control). However, the presence of formate did not significantly alter the percentage distribution of carbon-14 in the various fractions isolated. Of the organic acids labelled (Table 35), glycollate and malate together, contained a greater amount of carbon-14 as compared to the control. Additions of formate also reduced the amounts of carbon-14 entering the 3 position of serine (Table 36).

Glyoxylate was therefore extensively metabolized by the carrot tissues to produce a variety of products, particularly glycine and serine. In further experiments, actively growing tissues such as elongating roots, coleoptiles and young leaves were fed glyoxylate- $C^{14}$  to ascertain whether this compound would be utilized for the biosynthesis of glycine and serine.

### Excised Root Tips

#### 1. Pea Roots

The tips of 3 day old roots were excised and incubated with glyoxylate- $C^{14}$  for six hours. The results are shown in Table 32. Carbon-14 was recovered in glycine, serine



TABLE 32

The Metabolism of glyoxylate-1,2-C<sup>14</sup> by excised root tips

0.5 g of root tips (5 mm) incubated at 30° for 6 hr. in darkness with 100 μmoles of phosphate buffer (pH 5.6) and 1 μmole of glyoxylate containing 1 μc of C<sup>14</sup>.

| Fraction                 | Pea roots |                       | Corn roots |                       |
|--------------------------|-----------|-----------------------|------------|-----------------------|
|                          | C14 (cpm) | % of incorporated C14 | C14 (cpm)  | % of incorporated C14 |
| Carbon Dioxide           | 55,600    | 24.2                  | 60,000     | 30.4                  |
| Lipids                   | 3,200     | 1.4                   | 8,800      | 4.5                   |
| Sugars                   | 3,400     | 1.4                   | 10,400     | 5.2                   |
| Organic Acids            | 87,200    | 38.0                  | 16,700     | 8.4                   |
| Amino Acids              |           |                       |            |                       |
| Glycine                  | 14,500    | 6.3                   | 29,000     | 14.7                  |
| Serine                   | 12,400    | 5.4                   | 19,000     | 9.9                   |
| Glutamic & Aspartic acid | 20,200    | 8.8                   | 13,400     | 7.0                   |
| Others                   | 10,200    | 4.4                   | ...        | ...                   |
| Residue                  | 22,600    | 9.8                   | 39,500     | 20.5                  |
| Total C14 incorporated   | 229,000   |                       | 197,000    |                       |







and other fractions, including carbon dioxide and organic acids. Of the organic acid fraction, most of the radioactivity was present in glycollate and oxalate.

## 2. Corn Roots

Twenty-five percent of the carbon-14 incorporated from glyoxylate-C<sup>14</sup> was recovered in glycine and serine following a six hour experimental period with corn root tips. The organic acids were a minor fraction, consisting mainly of glycollate and oxalate. However, there was considerable release of carbon-14 as carbon dioxide, this alone accounting for 30.4% of the carbon-14 utilized (Table 32).

## 3. Corn Coleoptiles

Corn coleoptiles, in contrast to the excised roots, incorporated large amounts of carbon-14 into the organic acids (Table 33). This radioactivity was found to be due almost exclusively to glycollate and oxalate; only very small amounts of carbon-14 being incorporated into malate (Table 35). An unknown organic acid eluting in the glyceric acid fraction, also became labelled. In addition to labelling of the organic acids, glyoxylate was extensively incorporated into glycine and serine.

## 4. Pea Leaves

More than half of the carbon-14 incorporated was utilized in the synthesis of glycine and serine by the excised pea leaves in the dark. Carbon dioxide was again an important product, accounting for 23.1% of the carbon-14 activity incorporated. However, only relatively small amounts of carbon-14 entered the organic acids (Table 33).



TABLE 33

The Metabolism of glyoxylate-1,2-C14 by Corn coleoptiles and Pea leaves

0.5 g of tissue slices incubated at 30° for 6 hr. in darkness with 100  $\mu$ moles of phosphate buffer (pH 5.6) and 1  $\mu$ mole of glyoxylate containing 1  $\mu$ c C14.

| Fraction                 | Corn coleoptiles* |                       | Pea leaves** |                       |
|--------------------------|-------------------|-----------------------|--------------|-----------------------|
|                          | C14 (cpm)         | % of incorporated C14 | C14 (cpm)    | % of incorporated C14 |
| Carbon Dioxide           | 29,700            | 9.1                   | 103,200      | 23.1                  |
| Lipids                   | 1,000             | 0.3                   | 1,400        | 0.3                   |
| Sugars                   | 5,500             | 1.7                   | 16,000       | 3.7                   |
| Organic Acids            | 192,000           | 58.8                  | 20,700       | 4.6                   |
| Amino Acids              |                   |                       |              |                       |
| Glycine                  | 37,700            | 11.6                  | 170,000      | 38.1                  |
| Serine                   | 21,500            | 6.6                   | 63,200       | 14.2                  |
| Glutamic & Aspartic acid | 6,000             | 1.8                   | 1,800        | 0.4                   |
| Others                   | 10,500            | 3.2                   | 44,000       | 9.9                   |
| Residue                  | 22,600            | 6.9                   | 25,700       | 5.7                   |
| Total C14 incorporated   | 326,500           |                       | 446,000      |                       |

\* from 4 day old seedlings

\*\* from 15 day old seedlings



This fraction consisted of labelled glycollate, oxalate, malate, succinate, citrate and an unknown organic acid.

Degradations of serine, obtained from pea leaves, following glyoxylate- $C^{14}$  feeding, showed that most of the carbon-14 was located in the 1 position (Table 36). The 2 and 3 positions were almost equally labelled. However, the 3 position of serine contained 19.8% of the total carbon-14, as compared to only 4.7% in the carrot tissue. It is apparent, therefore, that the amounts of glyoxylate carbon entering the 3 position of serine varies with the tissue used in the experiments.

#### Germinating Seeds

In several species of germinating seeds, there is an extensive breakdown and resynthesis of proteins. In other species, where fat is the chief storage material, this might be utilized during germination for the synthesis of carbohydrates (Zeller 1957). In many fatty seeds, this conversion is presumed to take place via the partial reactions of the glyoxylate cycle. However, as shown by the feeding experiments, described above, glyoxylate may also be involved in the biosynthesis of certain amino acids in the tissues used. In order to test the possibility that this might also occur in fatty seeds, glyoxylate- $C^{14}$  was fed to germinating tissues known to contain the enzymes of the glyoxylate cycle.

#### Pea Cotyledons

Following a six hour incubation period with glyoxylate-1,2- $C^{14}$ , 3 day old pea cotyledons incorporated a large amount of carbon-14 into carbon dioxide (Table 34). The organic acids







TABLE 34

The Metabolism of glyoxylate-1,2-C14 by germinating seeds

0.5 g of cotyledon slices of sunflower (1 day old) and peas (3 day old) incubated at 30° for 6 hr. in darkness with 100  $\mu$ moles of phosphate buffer (pH 5.6) and 1  $\mu$ mole of glyoxylate containing 1  $\mu$ c of C14.

| Fraction                 | Pea cotyledons |                       | Sunflower cotyledons |                       |
|--------------------------|----------------|-----------------------|----------------------|-----------------------|
|                          | C14 (cpm)      | % of incorporated C14 | C14 (cpm)            | % of incorporated C14 |
| Carbon Dioxide           | 78,200         | 26.4                  | 55,000               | 14.7                  |
| Lipids                   | 3,300          | 1.1                   | 2,500                | 0.67                  |
| Sugars                   | 3,000          | 1.0                   | 9,000                | 2.4                   |
| Organic Acids            | 118,700        | 40.0                  | 67,500               | 18.1                  |
| Amino Acids              |                |                       |                      |                       |
| Glycine                  | 22,600         | 7.6                   | 39,600               | 10.6                  |
| Serine                   | 8,300          | 2.8                   | 39,800               | 10.7                  |
| Glutamic & Aspartic acid | 25,400         | 8.5                   | 47,000               | 12.7                  |
| Others                   | 14,000         | 4.7                   | 17,000               | 4.5                   |
| Residue                  | 32,600         | 7.6                   | 55,000               | 14.7                  |
| Total C14 incorporated   | 296,100        |                       | 371,600              |                       |



accounted for 40.0% of the total carbon-14 recovered, while glycine and serine together contained 10.4% of the carbon-14. As is evident from Table 35, the organic acid fraction mainly consisted of glycollate, although malate was also labelled.

#### Watermelon Cotyledons

Three and five day old watermelon cotyledons were supplied with glyoxylate- $C^{14}$  as shown in Table 37. In both experiments, glycine was the chief product of glyoxylate metabolism. In the five day old cotyledons, there was an increase in the amounts of carbon-14 being incorporated into serine. Incorporation of glyoxylate-carbon into the sugar fraction was in all cases very slight.

#### Sunflower Cotyledons

Glyoxylate-1,2- $C^{14}$  metabolism was followed in germinating sunflower cotyledons, excised from 1 to 5 day old seedlings. As is evident from Tables 38a and 38b, carbon dioxide was the major product of glyoxylate metabolism at all stages of germination. The organic acid fraction, consisting mainly of glycollate (Table 39), was an important product in the early stages of germination. However, throughout the germination period, glycine and serine contained large percentages of carbon-14. The sugar fraction contained the greatest percentage of carbon-14 when the cotyledons were 4 days old. At this stage of germination, the production of carbon dioxide was also maximal (44.6%), while the incorporation of carbon-14 into the organic acids was minimal (9.6%). The organic acid fraction was further analyzed and found to contain labelled glycollate and oxalate. Although malate- $C^{14}$  and citrate- $C^{14}$



TABLE 35

The Metabolism of glyoxylate-1,2-C<sup>14</sup> by plant tissues, organic acid fraction

Experimental procedure as described in tables 30-34. Results expressed as percentage of C<sup>14</sup> incorporated into the organic acid fraction.

| Organic<br>Acids     | Pea<br>Leaves | Sunflower<br>Cotyledons | Corn        |  | Pea<br>Cotyledons | Corn<br>Roots | Carrot Storage Tissue          |                                                            |
|----------------------|---------------|-------------------------|-------------|--|-------------------|---------------|--------------------------------|------------------------------------------------------------|
|                      |               |                         | Coleoptiles |  |                   |               | Glyoxylate-1,2-C <sup>14</sup> | Glyoxylate-1,2-C <sup>14</sup><br>+ 10 $\mu$ moles formate |
| Glycollate           | 18.1          | 25.1                    | 80.2        |  | 55.2              | 62.3          | 8.0                            | 18.1                                                       |
| Oxalate              | 13.0          | 55.5                    | 8.1         |  | 7.6               | 16.1          | 17.0                           | 8.9                                                        |
| Malate               | 6.3           | 4.5                     | 3.8         |  | 24.9              | 8.4           | 23.9                           | 29.7                                                       |
| Succinate            | 5.6           | -                       | -           |  | 9.9               | 13.2          | 23.9                           | 18.9                                                       |
| Citrate              | 21.2          | 14.9                    | -           |  | 2.3               | -             | 6.7                            | 6.4                                                        |
| Glucose<br>phosphate | -             | -                       | -           |  | -                 | -             | 13.6                           | 12.5                                                       |
| Pyruvate             | -             | -                       | -           |  | -                 | -             | 6.7                            | 5.5                                                        |
| Others               | 35.7          | -                       | 7.8         |  | -                 | -             | -                              | -                                                          |





TABLE 36

The Intramolecular distribution of Cl4 in Serine following feeding with glyoxylate-1,2-Cl4

Samples of serine-Cl4 recovered from tissues after 6 hr. glyoxylate-1,2-Cl4 metabolism were de-graded using periodate as described in Methods Section. Expressed as percentage of Cl4 recovered from products of degradation procedures.

| Carbon Number          |   | Carrot Storage Tissue |                                           | Sunflower<br>Cotyledons* |                    | Pea Leaves** |
|------------------------|---|-----------------------|-------------------------------------------|--------------------------|--------------------|--------------|
|                        |   | Glyoxylate-1,2-Cl4    | Glyoxylate-1,2-Cl4<br>+ 10 umoles formate | Glyoxylate-1,2-Cl4       | Glyoxylate-1,2-Cl4 |              |
| COOH                   | 1 | 52.8                  | 54.5                                      | 33.3                     |                    | 60.8         |
| <br>CHNH <sub>2</sub>  | 2 | 42.4                  | 43.3                                      | 38.8                     |                    | 19.3         |
| <br>CH <sub>2</sub> OH | 3 | 4.7                   | 2.1                                       | 27.7                     |                    | 19.8         |

\* 1 day old

\*\* excised from 15 day old seedlings



TABLE 37

The Metabolism of glyoxylate-1,2-C<sup>14</sup> by germinating watermelon cotyledons

0.5 g cotyledon slices incubated at 30° for 6 hr. in darkness with 100 μmoles of phosphate buffer (pH 5.6) and 1 μmole of glyoxylate containing 2 μc of C<sup>14</sup>.

| Fractions                          | 3 day old             |                                   | 5 day old             |                                   |
|------------------------------------|-----------------------|-----------------------------------|-----------------------|-----------------------------------|
|                                    | C <sup>14</sup> (cpm) | % of C <sup>14</sup> incorporated | C <sup>14</sup> (cpm) | % of C <sup>14</sup> incorporated |
| Carbon Dioxide                     | 222,000               | 31.9                              | 200,000               | 27.9                              |
| Sugars                             | 1,500                 | 0.2                               | 11,000                | 1.5                               |
| Organic Acids                      | 70,000                | 9.9                               | 110,000               | 15.4                              |
| Amino Acids                        |                       |                                   |                       |                                   |
| Glycine                            | 275,500               | 39.0                              | 228,000               | 31.9                              |
| Serine                             | 21,700                | 3.1                               | 95,000                | 13.3                              |
| Glutamic & Aspartic acid           | 35,300                | 5.0                               | 24,000                | 3.3                               |
| Residue                            | 80,000                | 11.3                              | 47,000                | 6.6                               |
| Total C <sup>14</sup> incorporated | 706,000               |                                   | 715,000               |                                   |



TABLE 38a

The Metabolism of glyoxylate-1,2-Cl<sup>14</sup> by germinating Sunflower cotyledons

0.5 g slices of cotyledons incubated at 30° for 6 hr. in darkness with 100 μmoles of phosphate buffer (pH 5.6) and 1 μmole of glyoxylate containing 1 μc of Cl<sup>14</sup>. Radioactivity expressed as cpm.

| Fractions                           | Age of Cotyledons (days) |         |         |         |         |
|-------------------------------------|--------------------------|---------|---------|---------|---------|
|                                     | 1                        | 2       | 3       | 4       | 5       |
| Carbon Dioxide                      | 94,000                   | 116,000 | 105,000 | 144,000 | 124,000 |
| Lipids                              | 2,500                    | 600     | 900     | 1,000   | 1,300   |
| Sugars                              | 9,000                    | 6,100   | 19,000  | 31,000  | 17,700  |
| Organic Acids                       | 67,500                   | 64,000  | 55,000  | 31,000  | 49,000  |
| Amino Acids                         |                          |         |         |         |         |
| Glycine                             | 39,600                   | 41,600  | 65,000  | 42,500  | 60,000  |
| Serine                              | 39,800                   | 33,400  | 50,000  | 16,500  | 23,000  |
| Glutamic & Aspartic acid            | 47,000                   | 33,300  | 23,600  | 11,000  | 23,000  |
| Others                              | 17,000                   | -       | -       | -       | -       |
| Residue                             | 55,000                   | 39,000  | 26,500  | 46,000  | 40,000  |
| Total Cl <sup>14</sup> incorporated | 371,000                  | 334,000 | 345,000 | 323,000 | 338,000 |





TABLE 38b

The Metabolism of glyoxylate-1,2-C<sup>14</sup> by germinating Sunflower cotyledons

Experimental procedure as in table 38a. Results expressed as percentage of total C<sup>14</sup> incorporated.

| Fraction                 | Age of Cotyledons (days) |      |      |      |      |
|--------------------------|--------------------------|------|------|------|------|
|                          | 1                        | 2    | 3    | 4    | 5    |
| Carbon Dioxide           | 25.3                     | 34.7 | 30.4 | 44.6 | 36.7 |
| Lipids                   | 0.7                      | 0.2  | 0.2  | 0.3  | 0.4  |
| Sugars                   | 2.4                      | 1.8  | 5.5  | 9.6  | 5.2  |
| Organic Acids            | 18.0                     | 19.0 | 16.0 | 9.6  | 14.5 |
| Amino Acids              |                          |      |      |      |      |
| Glycine                  | 10.7                     | 12.4 | 18.8 | 13.1 | 17.7 |
| Serine                   | 10.7                     | 10.0 | 14.6 | 5.1  | 6.8  |
| Glutamic & Aspartic acid | 12.7                     | 10.0 | 6.8  | 3.4  | 6.8  |
| Others                   | 4.6                      | -    | -    | -    | -    |
| Residue                  | 14.8                     | 11.6 | 7.7  | 14.2 | 11.8 |



TABLE 39

The Metabolism of glyoxylate-1,2- $\text{C}^{14}$  by germinating Sunflower cotyledons, organic acid fraction

Experimental procedure as in table 38a. Results expressed as percentage of  $\text{C}^{14}$  in organic acid fraction.

| <u>Organic Acids</u> | <u>Age of Cotyledons (days)</u> |          |          |
|----------------------|---------------------------------|----------|----------|
|                      | <u>1</u>                        | <u>3</u> | <u>5</u> |
| Glycollate           | 25.1                            | 77.8     | 80.6     |
| Malate               | 4.5                             | 10.2     | 6.2      |
| Citrate              | 14.8                            | 8.8      | 6.8      |
| Oxalate              | 55.5                            | 3.1      | 6.2      |



were detected, the amounts of radioactivity present were very low.

It is clear, therefore, that the sunflower cotyledons readily metabolized glyoxylate over the 5 day period of germination. Although there was a significant incorporation of glyoxylate into the sugars, this fraction contained considerably less carbon-14 than either glycine or serine. Clearly, glyoxylate was involved in amino acid biosynthesis to a considerable extent and only played a minor role in the biosynthesis of carbohydrate.

#### Linseed Cotyledons

Two day old linseed cotyledons, which are known to contain the enzymes of the glyoxylate cycle (Carpenter and Beevers 1959, Yamamoto and Beevers 1960), were incubated with glyoxylate- $C^{14}$  for a six hour period. As in the other germinating fatty seeds investigated, these tissues also converted glyoxylate into glycine, carbon dioxide and the insoluble residue. Appreciable amounts of radioactivity were also recovered in the organic acids and in serine. However, the sugar fraction contained only small amounts of carbon-14.

In another experiment (Table 40), non-radioactive acetate was added to the tissues during glyoxylate- $C^{14}$  feeding. If a large proportion of the glyoxylate metabolized was converted to malate via malate synthetase, such additions might increase the amounts of carbon-14 incorporated and possibly alter the distribution of carbon-14 in the products isolated. As is evident from Table 40, additions of acetate did result in a slight increase in the total carbon-14 incorporated.





TABLE 40

The Metabolism of glyoxylate-1,2-C<sup>14</sup> by germinating Linseed cotyledons\*

0.5 g cotyledon slices incubated at 30° for 6 hr. in darkness with 100 μmoles of phosphate buffer (pH 5.6) and 1 μmole of glyoxylate containing 1 μc of C<sup>14</sup>.

| Fractions                          | glyoxylate-1,2-C <sup>14</sup> |                                         | glyoxylate-1,2-C <sup>14</sup> +<br>5 μmoles acetate |                                         |
|------------------------------------|--------------------------------|-----------------------------------------|------------------------------------------------------|-----------------------------------------|
|                                    | <u>C<sup>14</sup> (cpm)</u>    | <u>% of incorporated C<sup>14</sup></u> | <u>C<sup>14</sup> (cpm)</u>                          | <u>% of incorporated C<sup>14</sup></u> |
| Carbon Dioxide                     | 55,000                         | 18.8                                    | 41,000                                               | 12.3                                    |
| Lipids                             | 1,000                          | 0.3                                     | 500                                                  | 0.1                                     |
| Sugars                             | 7,600                          | 2.6                                     | 4,700                                                | 1.4                                     |
| Organic Acids                      | 43,200                         | 14.7                                    | 67,500                                               | 20.3                                    |
| Amino Acids                        |                                |                                         |                                                      |                                         |
| Glycine                            | 104,000                        | 35.5                                    | 138,500                                              | 41.6                                    |
| Serine                             | 20,000                         | 6.8                                     | 18,000                                               | 5.4                                     |
| Glutamic & Aspartic                | 13,600                         | 4.6                                     | 11,800                                               | 3.5                                     |
| Residue                            | 48,600                         | 16.6                                    | 51,000                                               | 15.3                                    |
| Total C <sup>14</sup> incorporated | 293,000                        |                                         | 333,000                                              |                                         |

\* 2 day old



Although the amounts of glyoxylate carbon entering the respired carbon dioxide were reduced in the presence of acetate, there was a striking increase in the radioactivity present in the organic acids and in glycine.

It is clear, therefore, that additions of acetate did affect the extent of glyoxylate metabolism in these tissues.

#### Pumpkin Cotyledons

Three day old pumpkin cotyledons (Table 41), readily metabolized glyoxylate- $C^{14}$  and, as in the experiments with the other fatty seeds, glycine was the chief product of this metabolism. Other important products were carbon dioxide and the insoluble residue. The sugars and organic acids contained small amounts of carbon-14 and, therefore, only accounted for a minor fraction of the carbon-14 incorporated. Separation of the organic acid fraction (Table 42) showed that the major radioactive component was glycollate.

Unlike the experiments conducted with linseed cotyledons, the metabolism of glyoxylate by the pumpkin seedlings was not stimulated by additions of acetate (Table 41). In fact, additions of acetate decreased the amounts of glyoxylate carbon being incorporated into glycine and carbon dioxide.

The results obtained with pumpkin tissues are, therefore, similar to those obtained from experiments with the other fatty tissues. Glyoxylate was mainly incorporated into the amino acids. However, in this material, the metabolism of glyoxylate was not stimulated by additions of acetate.



TABLE 41

The Metabolism of glyoxylate-1,2-C14 by germinating Pumpkin cotyledons\*

0.5 g cotyledon slices incubated at 30° for 6 hr. in darkness with 100 μmoles of phosphate buffer (pH 5.6) and 1 μmole of glyoxylate containing 1 μc of C14.

| Fractions              | glyoxylate-1,2-C14 |                       | glyoxylate-1,2-C14 +<br>5 μmoles acetate |                       |
|------------------------|--------------------|-----------------------|------------------------------------------|-----------------------|
|                        | C14 (cpm)          | % of incorporated C14 | C14 (cpm)                                | % of incorporated C14 |
| Carbon Dioxide         | 74,000             | 20.8                  | 64,000                                   | 23.5                  |
| Lipids                 | 500                | 0.1                   | not active                               | -                     |
| Sugars                 | 4,800              | 1.3                   | 3,600                                    | 1.3                   |
| Organic Acids          | 24,000             | 6.7                   | 24,000                                   | 8.8                   |
| Amino Acids            |                    |                       |                                          |                       |
| Glycine                | 131,000            | 36.9                  | 110,600                                  | 40.5                  |
| Serine                 | 29,000             | 8.1                   | 25,000                                   | 9.1                   |
| Glutamic & Aspartic    | 14,000             | 4.0                   | 11,000                                   | 4.0                   |
| Residue                | 78,700             | 22.1                  | 34,800                                   | 12.7                  |
| Total C14 incorporated | 356,000            |                       | 273,000                                  |                       |

\* 3 day old





TABLE 42

The Effect of acetate on glyoxylate-1,2-Cl<sup>14</sup> metabolism by germinating Pumpkin cotyledons\*, organic acid fraction

Experimental procedure as in table 41.

| Organic Acids                                           | Glyoxylate-1,2-Cl <sup>14</sup> |                                                         | Glyoxylate-1,2-Cl <sup>14</sup> +<br>5 $\mu$ moles acetate |                                                         |
|---------------------------------------------------------|---------------------------------|---------------------------------------------------------|------------------------------------------------------------|---------------------------------------------------------|
|                                                         | Cl <sup>14</sup> (cpm)          | % of Cl <sup>14</sup> of total<br>organic acid fraction | Cl <sup>14</sup> (cpm)                                     | % of Cl <sup>14</sup> of total<br>organic acid fraction |
| Glycollate                                              | 15,000                          | 62.5                                                    | 13,600                                                     | 56.7                                                    |
| Oxalate                                                 | not active                      | -                                                       | 3,100                                                      | 12.9                                                    |
| Succinate                                               | 1,600                           | 6.7                                                     | 1,100                                                      | 4.6                                                     |
| Malate                                                  | 1,500                           | 6.2                                                     | 1,500                                                      | 6.2                                                     |
| Citrate                                                 | 2,700                           | 11.2                                                    | 2,700                                                      | 11.2                                                    |
| Glucose phosphate                                       | 900                             | 3.7                                                     | 800                                                        | 3.3                                                     |
| Others                                                  | 2,300                           | 9.6                                                     | 1,200                                                      | 5.0                                                     |
| Total Cl <sup>14</sup> incorporated<br>in organic acids | 24,000                          |                                                         | 24,000                                                     |                                                         |

\* 2 day old



## Glyoxylate Transaminase in Plant Tissues

As was shown earlier, glycine-C<sup>14</sup> was a major component of the soluble fraction following glyoxylate-C<sup>14</sup> feeding in all the plant tissues examined. Glycine might conceivably arise from glyoxylate, by addition of an amino group in a transaminase reaction. Studies with *Blastocladiella* (McCurdy and Cantino 1960), *Neurospora* (Campbell 1956) and *Phaseolus radiatus* (Sastry and Ramakrishnan 1961), have indicated the existence of such a glyoxylate transaminase system. In addition, data derived from several sources (Canvin and Beevers 1961, Wang and Waygood 1962), might be interpreted as indicating that such a system also exists in a variety of higher plant tissues. In order to ascertain whether the present tissues contained an enzyme system capable of synthesizing glycine from glyoxylate, extracts were prepared as described in the Methods Section. In all cases, glyoxylate-transaminase activity was measured by the ability of the extracts to convert glyoxylate-C<sup>14</sup> into glycine-C<sup>14</sup> in the presence of a suitable amino donor. Extracts for assay of glyoxylate-transaminase activity were prepared from a variety of higher plant tissues, including those known to convert glyoxylate into glycine in vivo. In addition to this survey of the occurrence of this enzyme in plants, tissues particularly rich in the enzyme, were used in studies on the properties and intracellular distribution of the transaminase.

## Occurrence of Glyoxylate Transaminase in Various Plant Tissues

### 1. Carrot Tissues

In carrot tissues, glyoxylate-transaminase activity



TABLE 43

Glyoxylate-glycine transaminase activity of Carrot storage tissue

Each reaction flask contained: 2 ml dialyzed enzyme in 0.1M phosphate buffer (pH 7.2); 20 µg pyridoxal phosphate; 0.5 µmoles of glyoxylate-1,2-Cl<sup>14</sup> (12 x 10<sup>4</sup> cpm) in a total volume of 2.45 mls incubated at 30° for 4 hr. in nitrogen. Additions of DL amino acids (2 µmoles) were made as indicated.

| Treatment              | Glycine-Cl <sup>14</sup> synthesized/hr. |                  | Glycine-Cl <sup>14</sup> synthesized<br>(%) over control |
|------------------------|------------------------------------------|------------------|----------------------------------------------------------|
|                        | cpm/g.f. wt                              | cpm/mg protein N |                                                          |
| Enzyme - control       | 29,970                                   | 3,500            | 100                                                      |
| Enzyme + alanine       | 66,060                                   | 7,700            | 220                                                      |
| Enzyme + glutamic acid | 49,440                                   | 5,660            | 160                                                      |
| Enzyme + serine        | 39,400                                   | 4,600            | 131                                                      |
| Enzyme + aspartic acid | 35,440                                   | 4,120            | 118                                                      |





TABLE 44

Glyoxylate-glycine transaminase activity of 7 day old etiolated Corn coleoptiles

Each reaction flask contained: 2 mls dialyzed enzyme in 0.1M phosphate buffer (pH 7.2); 20 µg pyridoxal phosphate; 0.5 µmoles glyoxylate-1,2-Cl<sup>14</sup> (12 x 10<sup>4</sup> cpm) in a total volume of 2.45 mls incubated at 300 for 4 hr. in nitrogen. Additions of DL amino acids (2 µmoles) were made as indicated.

| Treatment              | Glycine-Cl <sup>14</sup> synthesized/hr. |                  | Glycine-Cl <sup>14</sup> synthesized<br>(%) over control |
|------------------------|------------------------------------------|------------------|----------------------------------------------------------|
|                        | cpm/g.f. wt                              | cpm/mg protein N |                                                          |
| Enzyme - control       | 15,500                                   | 11,075           | 100                                                      |
| Enzyme + alanine       | 76,500                                   | 54,650           | 493                                                      |
| Enzyme + aspartic acid | 75,500                                   | 53,925           | 487                                                      |
| Enzyme + glutamic acid | 68,000                                   | 48,575           | 439                                                      |
| Enzyme + serine        | 48,500                                   | 34,650           | 313                                                      |



TABLE 45

The Effect of various amino donors on the glyoxylate-glycine transaminase activity of excised pea leaves\*

Each reaction flask contained: 1 ml of dialyzed enzyme in 0.1M phosphate buffer (pH 7.2); 20 µg pyridoxal phosphate; 1 µmole glyoxylate-1,2-C<sup>14</sup> (4.8 x 10<sup>4</sup> cpm) in a total volume of 1.45 mls incubated at 30° for 90 minutes in nitrogen. Additions of 2 µmoles of amino donors were made in the treatments.

| Treatment                  | Glycine-C <sup>14</sup> synthesized/hr. |                  | Glycine-C <sup>14</sup> synthesized<br>(%) over control |
|----------------------------|-----------------------------------------|------------------|---------------------------------------------------------|
|                            | cpm/g.f. wt                             | cpm/mg protein N |                                                         |
| Enzyme - control           | 28,260                                  | 6,670            | 100                                                     |
| Enzyme + DL glutamic acid  | 95,200                                  | 24,260           | 364                                                     |
| Enzyme + DL alanine        | 88,000                                  | 20,940           | 314                                                     |
| Enzyme + DL aspartic acid  | 82,600                                  | 19,660           | 295                                                     |
| Enzyme + DL serine         | 82,000                                  | 19,640           | 295                                                     |
| Enzyme + DL threonine      | 50,600                                  | 12,000           | 180                                                     |
| Enzyme + DL tryptophane    | 44,000                                  | 10,660           | 160                                                     |
| Enzyme + DL phenylalanine  | 38,600                                  | 9,200            | 138                                                     |
| Enzyme + ammonium sulphate | 31,160                                  | 7,260            | 109                                                     |
| Enzyme + hydroxylamine     | 2,000                                   | 476              | 7                                                       |

\* excised from 15 day old seedlings



was assayed using alanine, glutamic acid, serine and aspartic acid as amino donors (Table 43). Alanine proved to be the best amino donor, being followed by glutamic acid, serine and aspartic acid in order of their ability to stimulate glycine formation over the control.

## 2. Corn Coleoptiles

Extracts prepared from etiolated corn coleoptiles (Table 44) contained large amounts of glyoxylate transaminase. Alanine was again the most efficient amino donor of those tested. However, there was also marked stimulation in glycine production when glutamic and aspartic acids were present. Since the glyoxylate- $C^{14}$  solution used in this experiment had the same specific activity as that used for the carrot tissue extracts, it is clear that the corn coleoptiles contained considerably greater amounts of glyoxylate transaminase.

## 3. Pea Leaves

In studies on glyoxylate transaminase present in excised pea leaves, the efficiency of a large number of amino donors was investigated (Table 45). It is clear from Table 45, that the pea leaves contained an active glyoxylate transaminase system. Glutamic acid proved to be the best amino donor, being closely followed by alanine. The aromatic amino acids, phenylalanine and tryptophane, gave smaller stimulation than the aliphatic amino acids tested and inorganic forms of nitrogen, such as ammonium sulphate and hydroxylamine, were not utilized in the transamination reaction. Apparently, the enzyme can utilize the amino







groups of the various amino donors tested, but with varying efficiency.

#### 4. Sunflower Cotyledons

In sunflower cotyledons, changes in the activity of glyoxylate transaminase were studied during germination (Table 46). It is evident from Table 46, that additions of glutamic acid and alanine stimulated the production of glycine in extracts prepared from one day old cotyledons. This stimulation was greatest when glutamic acid was present as the amino donor. After 48 hours, the efficiency of glutamic acid as an amino donor decreased, whereas the efficiency of alanine was further increased. This was followed by further decreases in the amounts of stimulation caused by glutamic acid and alanine over the remaining experimental period. It can therefore be concluded that the activity of glyoxylate transaminase changes considerably during germination, being most active in the youngest cotyledons. Furthermore, changes in the relative efficiency of glutamic acid and alanine as amino donors might indicate the existence of separate glyoxylate:glutamate and glyoxylate:alanine transaminase systems in these tissues.

#### Properties of Glyoxylate Transaminase from Pea Leaves

Extracts were prepared from excised pea leaves and used in a study of the properties of the glyoxylate transaminase system.

##### 1. The Effect of pH on Glyoxylate Transaminase Activity

Glyoxylate transaminase activity was assayed at



TABLE 46

Changes in the glyoxylate-glycine transaminase activity of cotyledons during the germination of Sunflower seeds

Each reaction flask contained: 1 ml of dialyzed enzyme in 0.1M phosphate buffer (pH 7.2), 20 µg pyridoxal phosphate, 1 µmole of glyoxylate-1,2-Cl<sup>4</sup> (4.8 x 10<sup>4</sup> cpm) incubated at 30° for 90 mins. in nitrogen. Additions of 2 µmoles DL alanine and DL glutamic acid were made as indicated.

| Treatment                                              | Age of Cotyledons (days) |         |        |        |        |
|--------------------------------------------------------|--------------------------|---------|--------|--------|--------|
|                                                        | 1                        | 2       | 3      | 4      | 5      |
| Enzyme : Control                                       |                          |         |        |        |        |
| Glycine-Cl <sup>4</sup> synthesized/hr.<br>cpm/g.f. wt | 24,250                   | 12,000  | 32,000 | 33,000 | 57,750 |
| cpm/mg Protein N                                       | 4,400                    | 770     | 1,320  | 1,000  | 2,700  |
| % over control                                         | 100                      | 100     | 100    | 100    | 100    |
| Enzyme + DL Alanine                                    |                          |         |        |        |        |
| Glycine-Cl <sup>4</sup> synthesized/hr.<br>cpm/g.f. wt | 95,500                   | 123,600 | 87,000 | 55,200 | 39,200 |
| cpm/mg Protein N                                       | 17,200                   | 7,920   | 3,530  | 1,670  | 1,830  |
| % over control                                         | 391                      | 1,028   | 267    | 167    | 68     |
| Enzyme + DL Glutamic Acid                              |                          |         |        |        |        |
| Glycine-Cl <sup>4</sup> synthesized/hr.<br>cpm/g.f. wt | 128,000                  | 30,300  | 40,200 | 33,600 | 58,800 |
| cpm/mg Protein N                                       | 21,400                   | 2,000   | 1,630  | 1,000  | 2,750  |
| % over control                                         | 486                      | 260     | 123    | 100    | 102    |



different pH values as indicated in Table 47. Glycine-C<sup>14</sup> synthesis was maximal at pH 5.6 and 8.6.

## 2. Inhibition of Glyoxylate Transaminase Activity with Hydroxylamine

As many transaminase systems require pyridoxal phosphate as a co-enzyme, they are strongly inhibited by compounds such as hydroxylamine, which combines with the aldehyde group of the co-enzyme (Sakami and Harrington 1963, Meister 1956). In agreement with work published on transaminases from animals and microorganisms, the glyoxylate transaminase of pea leaves was strongly inhibited by hydroxylamine (Table 48). In the presence of 0.5  $\mu$ mole of hydroxylamine, the transaminase activity was inhibited by 21%. Increasing the hydroxylamine concentration resulted in further inhibition of enzyme activity.

## 3. Reversibility of Glyoxylate Transaminase

Reversibility of the glyoxylate transaminase, prepared from pea leaves, was studied using glyoxylate-C<sup>14</sup> and glycine-C<sup>14</sup> solutions in the presence of amino donors and amino acceptors as shown in Table 49. In the study of glycine synthesis, glyoxylate-C<sup>14</sup>, containing 66,000 cpm of carbon-14, was supplied. The results indicate, that in control, only 33% of the glyoxylate was converted into glycine-C<sup>14</sup>. In the presence of aspartic acid and alanine, however, 96% and 100% of the glyoxylate-C<sup>14</sup> was used in the synthesis of glycine during the experimental period.

In another experiment (Table 49), the production of glyoxylate-C<sup>14</sup> from glycine-C<sup>14</sup> in the presence of







TABLE 47

The Effect of pH on glyoxylate-glycine transaminase activity of excised Pea leaves\*

Each reaction flask contained: 0.5 ml dialyzed enzyme in 0.1M phosphate buffer (pH 7.2); 2  $\mu$ moles of DL glutamic acid; 20  $\mu$ g pyridoxal phosphate; 2 ml of phosphate buffer (pH as indicated below) and 1  $\mu$ mole of glyoxylate-1,2- $\text{Cl}^{14}$  ( $4.8 \times 10^4$  cpm) in a total volume of 2.9 mls incubated at 30° for 90 minutes in nitrogen.

| <u>pH</u> | <u>Glycine-<math>\text{Cl}^{14}</math> synthesized/hr.</u> |                         |
|-----------|------------------------------------------------------------|-------------------------|
|           | <u>cpm/g.f. wt</u>                                         | <u>cpm/mg protein N</u> |
| 4.6       | 87,740                                                     | 20,860                  |
| 5.6       | 102,000                                                    | 24,260                  |
| 6.6       | 94,600                                                     | 22,540                  |
| 7.6       | 90,600                                                     | 21,600                  |
| 8.6       | 102,600                                                    | 24,600                  |

\* excised from 15 day old seedlings



TABLE 48

The Inhibition of glyoxylate-glycine transaminase of excised Pea leaves\* by hydroxylamine

Each reaction flask contained: 1 ml dialyzed enzyme in 0.1M phosphate buffer (pH 7.2); 2  $\mu$ moles of DL glutamic acid and 1  $\mu$ mole of glyoxylate-1,2-Cl<sub>4</sub> (4.8 x 10<sup>4</sup> cpm) incubated at 30° for 1 hr. in nitrogen. Additions of hydroxylamine and pyridoxal phosphate were made as indicated.

| Treatment                                                   | Glycine-Cl <sub>4</sub> synthesized/hr. |                  | Glycine-Cl <sub>4</sub> synthesized<br>(%) over control |
|-------------------------------------------------------------|-----------------------------------------|------------------|---------------------------------------------------------|
|                                                             | cpm/g.f. wt                             | cpm/mg protein N |                                                         |
| Enzyme - control                                            | 48,000                                  | 11,400           | 100                                                     |
| Enzyme + 0.5 $\mu$ moles hydroxylamine + 0.5 $\mu$ moles PP | 38,000                                  | 9,000            | 79.0                                                    |
| Enzyme + 5 $\mu$ moles hydroxylamine + 0.5 $\mu$ moles PP   | 24,000                                  | 5,700            | 50.0                                                    |
| Enzyme + 10 $\mu$ moles hydroxylamine + 0.5 $\mu$ moles PP  | 20,000                                  | 4,760            | 41.7                                                    |
| Enzyme + 2 $\mu$ moles hydroxylamine                        | 21,200                                  | 5,050            | 44.3                                                    |
| Enzyme + 5 $\mu$ moles PP + 0.5 $\mu$ moles hydroxylamine   | 24,000                                  | 5,700            | 50.0                                                    |

\* excised from 15 day old seedlings

PP Pyridoxal Phosphate



TABLE 49

The Reversibility of the glyoxylate-glycine transaminase system of pea leaves

Glyoxylate to glycine:

Each reaction flask contained: 1 ml of dialyzed enzyme in 0.1M phosphate buffer (pH 7.2), 20 µg pyridoxal phosphate and 1 µmole of glyoxylate containing 0.2 µc of Cl<sup>14</sup> in total volume of 1.4 ml, incubated at 30° for 90 minutes in nitrogen. Additions of 2 µmoles of amino acids were made as indicated.

| Treatments                | Glycine-Cl <sup>14</sup> synthesized |                  |
|---------------------------|--------------------------------------|------------------|
|                           | cpm/g.f.wt.                          | cpm/mg Protein N |
| Enzyme: control           | 42,400                               | 10,000           |
| Enzyme + DL aspartic acid | 124,000                              | 29,500           |
| Enzyme + DL alanine       | 132,000                              | 31,400           |

\* \* \*

Glycine to glyoxylate:

Each reaction flask contained: 1 ml of dialyzed enzyme in 0.1M phosphate buffer (pH 7.2), 20 µg pyridoxal phosphate and 1 µmole of glycine-2-Cl<sup>14</sup> with 0.5 µc of Cl<sup>14</sup> in total volume of 1.4 ml, incubated at 30° for 90 minutes. Additions of 2 µmoles of keto acids were made as indicated.

| Treatments            | Glyoxylate-Cl <sup>14</sup> synthesized |                  |
|-----------------------|-----------------------------------------|------------------|
|                       | cpm/g.f.wt.                             | cpm/mg Protein N |
| Enzyme: control       | 9,660                                   | 2,340            |
| Enzyme + oxaloacetate | 13,340                                  | 3,180            |
| Enzyme + pyruvate     | 10,920                                  | 2,600            |



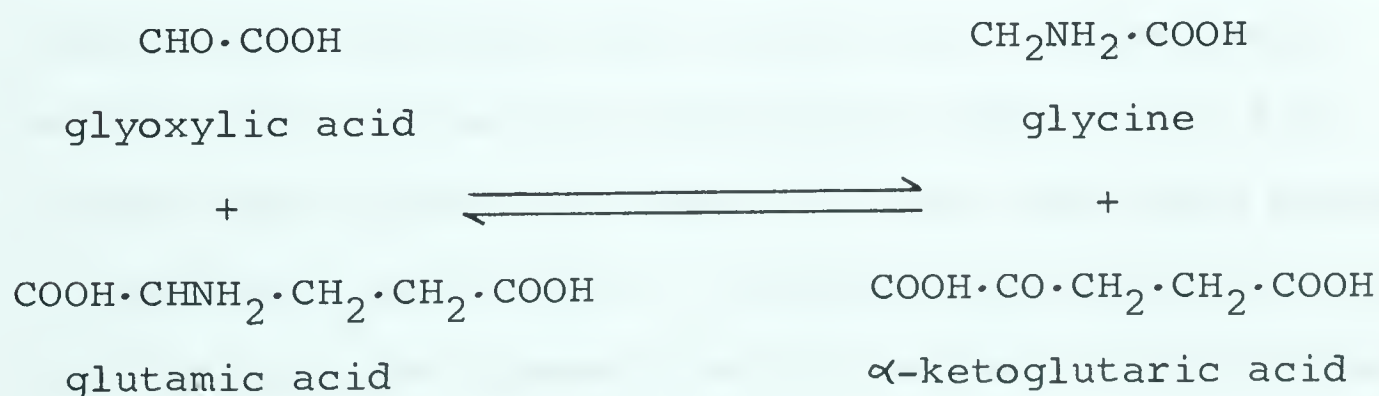


oxaloacetate and pyruvate was followed. In this experiment, glycine- $C^{14}$ , containing 150,000 cpm of carbon-14, was supplied. Only 6.0% of the glycine- $C^{14}$  was converted into glyoxylate in control, while in the presence of oxaloacetate and pyruvate, 9.0% and 7.3% of the glycine carbon was recovered in glyoxylate.

It is evident, therefore, that there was considerably more synthesis of glycine from glyoxylate than formation of glyoxylate in the reverse reaction. Apparently, under in vitro conditions, the glyoxylate transaminase system favors glycine synthesis.

#### 4. Coupling of Glyoxylate Transaminase with Glutamic Acid

In experiments with the glyoxylate transaminase of pea leaves, additions of glutamic acid led to increases in the amounts of glycine produced. If the transamination reaction proceeds as suggested in the equation (see below), the amino group of glycine would originate from the amino group of glutamic acid and  $\alpha$ -ketoglutaric acid would be produced.



In order to test this, glutamate- $C^{14}$  was added to extracts of pea leaves, known to produce glycine from



glyoxylate in the presence of glutamate. After a 90 minute experimental period, the extracts were analyzed for  $\alpha$ -ketoglutarate- $C^{14}$  (Table 50). As was expected, glutamate- $C^{14}$  was readily converted into  $\alpha$ -ketoglutarate.

Furthermore, this conversion was enhanced by 170% when 2  $\mu$ moles of glyoxylate were present in the reaction mixture.

5. The Rate of Product Formation During Glyoxylate Transamination

The rate of glycine- $C^{14}$  formation from glyoxylate- $C^{14}$  was studied using dialyzed homogenates prepared from pea leaves (fig. 1). In the controls, glycine was produced throughout the experimental period. However, in the presence of glutamic acid, glycine formation was enhanced with equilibrium being attained after 30 minutes.

6. Intracellular Localization of Glyoxylate Transaminase Activity in Sunflower Cotyledons

Glyoxylate- $C^{14}$  feeding experiments, with germinating fatty seeds, showed that carbon dioxide and glycine were the chief products. Since these tissues are known to contain the enzymes of the glyoxylate cycle (Marcus and Velasco 1959, Bradbeer and Stumpf 1959, Yamamoto and Beevers 1960, Carpenter and Beevers 1959), it is surprising that malate and sugars were not the chief products of glyoxylate metabolism. A possible explanation for this might be that the tissues contain enzymes for glyoxylate metabolism in addition to those of the glyoxylate cycle. For example, an active glyoxylate transaminase system might compete with malate synthetase for endogenous substrate.



TABLE 50

Coupling of the glyoxylate-glycine and  $\alpha$ -ketoglutarate-glutamate transaminase systems in pea leaf\* extracts

Each reaction flask contained: 1 ml of dialyzed enzyme in 0.1M phosphate buffer (pH 7.2), 20  $\mu$ g pyridoxal phosphate, 1  $\mu$ mole of glutamic acid containing 0.5  $\mu$ c of  $C^{14}$  in total volume of 1.4 ml incubated at 30° for 90 mins. in nitrogen.

| <u>Treatments</u>                    | <u><math>\alpha</math>-ketoglutarate-<math>C^{14}</math> Synthesized</u> |                          |                       |
|--------------------------------------|--------------------------------------------------------------------------|--------------------------|-----------------------|
|                                      | <u>cpm/g.f. wt</u>                                                       | <u>cpm/mg. Protein N</u> | <u>% over control</u> |
| Enzyme: control                      | 49,400                                                                   | 11,740                   | 100                   |
| Enzyme + 2 $\mu$ moles<br>glyoxylate | 133,400                                                                  | 31,740                   | 270                   |

\* excised from 15 day old seedlings

## Figure 1

### The Rate of Product Formation During Glyoxylate Transamination

Each reaction flask contained: 1 ml of dialyzed enzyme in 0.1M phosphate buffer (pH 7.2); 20  $\mu$ g pyridoxal phosphate; 1  $\mu$ mole glyoxylate-1,2- $C^{14}$  ( $4.8 \times 10^4$  cpm) in a total volume of 1.45 mls incubated at 30 $^{\circ}$  in nitrogen. Additions of 2  $\mu$ moles of DL glutamic acid were made as indicated.



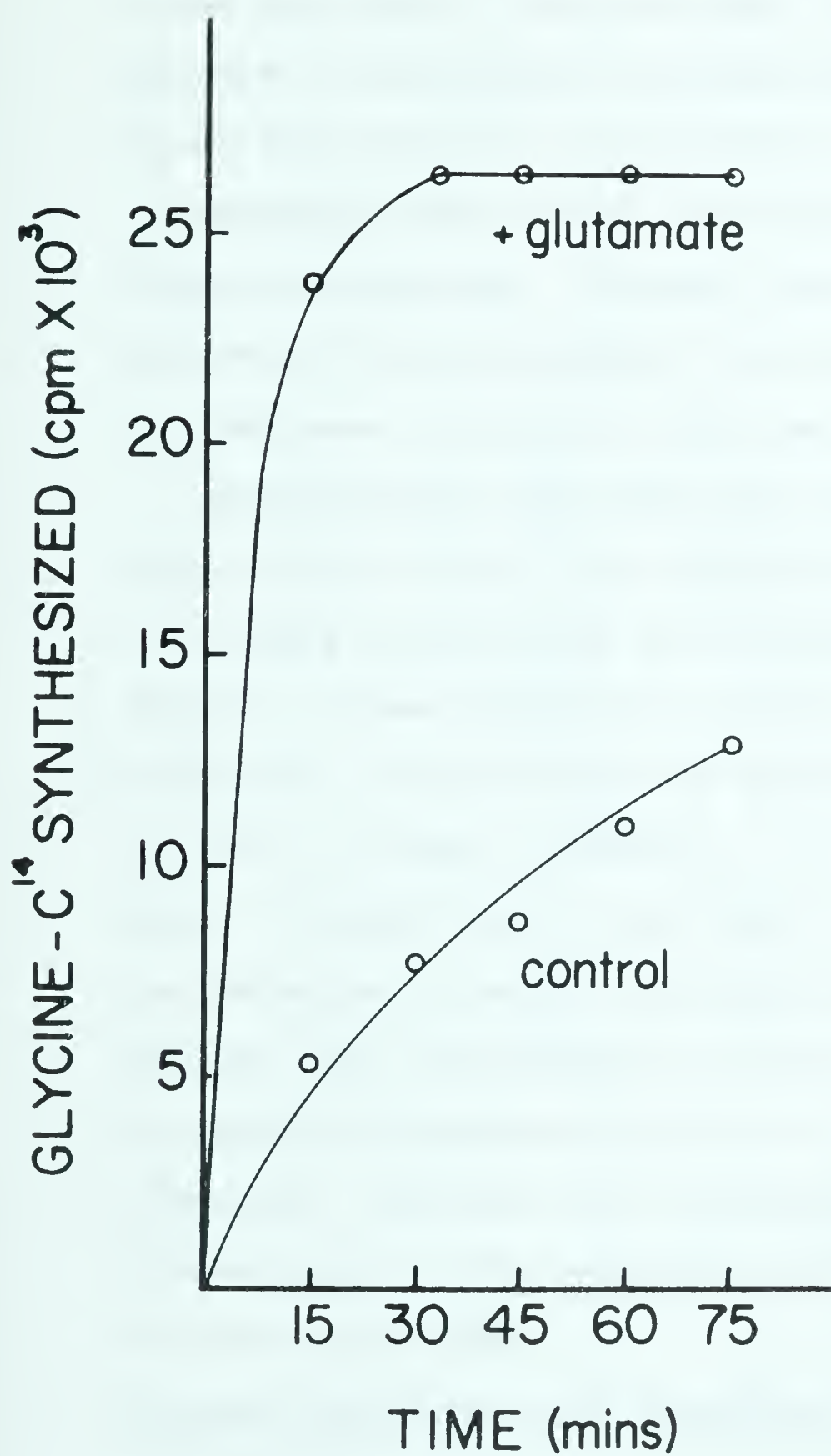


Figure 1

The Rate of Product Formation During Glyoxylate Transamination



Malate synthetase has been isolated and purified from mitochondrial extracts (Yamamoto and Beevers 1960) and is therefore presumed to be intracellularly localized in these particles. Alternatively, if glyoxylate transaminase is distributed throughout the cytoplasm and not localized within the mitochondria, it might compete very successfully with malate synthetase for exogenously supplied glyoxylate. Attempts were therefore made to determine the intracellular distribution of glyoxylate transaminase activity in sunflower cotyledons.

Mitochondrial and high speed supernatant fractions were obtained from 3 day old germinating sunflower cotyledons as described in the Materials and Methods Section. These fractions, together with the original crude homogenate, were assayed for glyoxylate-transaminase activity as shown in Table 51. It is evident, that on the basis of fresh weight, the bulk of the transaminase activity was recovered from the high speed supernatant fraction. However, the mitochondrial fraction contained some glyoxylate-transaminase activity. The present study, therefore, indicates that glyoxylate transaminase is more concentrated in the cytoplasm of sunflower cotyledons than in the mitochondria.

#### 7. Partial Purification of Glyoxylate Transaminase From Sunflower Cotyledons

It has been shown in the earlier experiments that glyoxylate-transaminase activity could be readily detected in homogenates prepared in dilute phosphate buffer. In



TABLE 51

Intracellular localization of glyoxylate-glycine transaminase activity in germinating Sunflower cotyledons\*

Each reaction flask contained: 1 ml enzyme in sucrose-phosphate buffer (pH 7.2); 20 µg of pyridoxal phosphate; 2 µmoles of amino donor as indicated and 1 µmole of glyoxylate-1,2-Cl<sup>4</sup> (12 x 10<sup>4</sup> cpm) incubated for 1 hr. at 30° in nitrogen.

| Treatment                       | Glycine-Cl <sup>4</sup> synthesized/hr. |                  | Glycine-Cl <sup>4</sup> synthesized<br>(%) over control |
|---------------------------------|-----------------------------------------|------------------|---------------------------------------------------------|
|                                 | cpm/g.f. wt                             | cpm/mg protein N |                                                         |
| Crude homogenate                |                                         |                  |                                                         |
| Enzyme - control                | 104,500                                 | 7,860            | 100                                                     |
| Enzyme + DL alanine             | 143,450                                 | 10,800           | 137                                                     |
| Enzyme + DL glutamic acid       | 89,490                                  | 6,730            | 87                                                      |
| High speed supernatant fraction |                                         |                  |                                                         |
| Enzyme - control                | 48,400                                  | 5,000            | 100                                                     |
| Enzyme + DL alanine             | 96,900                                  | 10,000           | 200                                                     |
| Enzyme + DL glutamic acid       | 64,500                                  | 6,500            | 130                                                     |
| Mitochondrial fraction          |                                         |                  |                                                         |
| Enzyme - control                | 1,790                                   | 2,240            | 100                                                     |
| Enzyme + DL alanine             | 5,700                                   | 7,140            | 320                                                     |
| Enzyme + DL glutamic acid       | 4,380                                   | 5,400            | 241                                                     |

\* 3 day old





further experiments, attempts were made to fractionate the protein present in these homogenates and therefore to partially purify the glyoxylate transaminase from the other proteins present. The results are summarized in Table 52. The greatest amount of glyoxylate-transaminase activity was recovered in the fraction precipitating at 75% saturation with ammonium sulphate. This fraction, besides containing the greatest number of enzyme units, contained the highest specific enzyme activity.

The experiments with cell-free extracts have demonstrated that glyoxylate transaminase is widespread in plant tissues. This enzyme system might, therefore, account for the large amounts of glycine synthesized from glyoxylate in vivo.



TABLE 52

Partial purification of glyoxylate-glycine transaminase from Sunflower cotyledons\*

Each reaction flask contained: 1 ml enzyme in 0.1M phosphate buffer (pH 7.2), 20 µg pyridoxal phosphate, 2 µmoles of DL glutamic acid and 1 µmole of glyoxylate containing 0.5 µc of C14 in a total volume of 1.3 ml, incubated at 30° for 1 hr. in nitrogen. Results expressed as units of enzyme\*\*.

| <u>Enzyme obtained from</u>                                       | <u>Units</u> | <u>mg protein N</u> | <u>Specific activity of enzyme</u><br><u>Units/mg protein N</u> | <u>Recovery</u><br><u>%</u> |
|-------------------------------------------------------------------|--------------|---------------------|-----------------------------------------------------------------|-----------------------------|
| Crude homogenate                                                  | 5890         | 1190                | 4.9                                                             | 100                         |
| 50% Saturation of (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> | 1490         | 364                 | 4.0                                                             | 23.6                        |
| 75% Saturation of (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> | 2180         | 272                 | 8.0                                                             | 37.0                        |
| 90% Saturation of (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> | 210          | 105                 | 2.0                                                             | 3.6                         |

\* 40 g of germinating 4 day old sunflower cotyledons

\*\* Unit of enzyme defined as the amount of enzyme incubated at 30° in nitrogen, producing 100 cpm of glycine-C14 in 60 seconds from 1 µmole of glyoxylate containing 0.5 µc of C14.



### Acetate Metabolism in Germinating Fatty Seeds

In the present investigation, exogenously supplied glyoxylate was mainly incorporated into glycine, carbon dioxide and the insoluble residue of germinating sunflower, pumpkin and linseed cotyledons. As the insoluble residue was found to contain large amounts of carbon-14 in protein amino acids (Table 68), it might be assumed that considerable amino acid and protein synthesis occurs in these tissues.

There is now considerable evidence (Zeller 1957) that the storage fat, present in seeds such as those of sunflower, is broken down during germination and is utilized in synthetic reactions occurring within the storage tissues. The utilization of acetyl-CoA, arising from fatty acid oxidation, in sugar biosynthesis, has received considerable attention recently. Work by Bradbeer and Stumpf and by Beevers' group (Beevers 1961a) has demonstrated the existence of a glyoxylate cycle by which these synthetic reactions are possible.

It is also theoretically possible, that in tissues containing an active glyoxylate cycle, the net synthesis of organic acids arising from cycle operation, might be utilized for the biosynthesis of amino acids. The present studies have indicated that amino acid biosynthesis occurs in germinating sunflower cotyledons, which are also known to contain the enzymes of the glyoxylate cycle. Investigations into the possible role of the glyoxylate cycle in amino acid biosynthesis were therefore conducted using acetate-2-C<sup>14</sup> solutions. In addition to these studies with sunflower tissues, similar investigations were conducted with other fatty seeds known to





contain the enzymes of the glyoxylate cycle.

### Acetate-2-C<sup>14</sup> Metabolism in Germinating Sunflower Cotyledons

#### 1. Products of Acetate Metabolism

Slices of germinating sunflower cotyledons were incubated with acetate-2-C<sup>14</sup> at various stages of germination as indicated in Table 53a. The greatest amounts of acetate carbon were utilized in the 1 day old tissues. As germination proceeded, there was a marked decrease in the total carbon-14 metabolized during the experimental period. Although the sugars were never a major product of acetate metabolism, the carbon-14 content of this fraction rose to a maximum after 5 days germination. Analysis of the organic acid fraction indicated that acetate was readily incorporated into the acids of the glyoxylate cycle during germination (Table 55). Malate was always a major radioactive component of the organic acid fraction, but glycollate contained increasing percentages of carbon-14 as germination proceeded. Radioactivity was detected in glyoxylate in the 3 day old cotyledons.

As is evident from Table 53a and 53b, the amino acid fractions contained the bulk of the carbon-14 at all stages of germination. The neutral and basic amino acid fraction (Table 54) contained large amounts of labelled glutamine and alanine, together with glycine,  $\gamma$ -amino butyric acid and asparagine.

It appears, therefore, that acetate was utilized throughout the germination period examined and that considerable amounts of acetate carbon are involved in the



TABLE 53a

The Metabolism of acetate-2-C14 by germinating Sunflower cotyledons

0.5 g slices of cotyledons incubated at 30° for 6 hr. in darkness with 100 μmoles of phosphate buffer (pH 5.6) and 1 μmole of acetate-2-C14 containing 5 μc of C14. Radioactivity expressed as cpm.

| Fractions                   | Age of Cotyledons (days) |           |           |         |         |         |
|-----------------------------|--------------------------|-----------|-----------|---------|---------|---------|
|                             | 1                        | 2         | 3         | 4       | 5       | 6       |
| Carbon Dioxide              | 166,000                  | 170,000   | 178,000   | 108,000 | 157,000 | 145,000 |
| Lipids                      | 12,000                   | 19,000    | 16,000    | 18,000  | 18,000  | 5,000   |
| Sugars                      | 85,000                   | 140,000   | 88,000    | 91,000  | 126,000 | 28,000  |
| Organic Acids               | 454,000                  | 262,000   | 116,000   | 102,000 | 116,000 | 132,000 |
| Amino Acids                 |                          |           |           |         |         |         |
| Neutral & Basic amino acids | 339,000                  | 552,000   | 564,000   | 335,000 | 319,000 | 269,000 |
| Acidic amino acids          | 600,000                  | 102,000   | 156,000   | 65,000  | 89,000  | 71,000  |
| Residue                     | 105,000                  | 170,000   | 143,000   | 83,000  | 97,000  | 76,000  |
| Total C14 incorporated      | 1,761,000                | 1,415,000 | 1,261,000 | 802,000 | 922,000 | 726,000 |



TABLE 53b

The Metabolism of acetate-2-C<sup>14</sup> by germinating Sunflower cotyledons

Experimental procedure as in table 53a. Results expressed as percentage of total C<sup>14</sup> incorporated.

| Fractions                   | Age of Cotyledons (days) |      |      |      |      |      |
|-----------------------------|--------------------------|------|------|------|------|------|
|                             | 1                        | 2    | 3    | 4    | 5    | 6    |
| Carbon Dioxide              | 9.4                      | 12.0 | 14.1 | 13.4 | 17.0 | 20.0 |
| Lipids                      | 0.7                      | 1.3  | 1.3  | 2.2  | 2.0  | 0.7  |
| Sugars                      | 4.8                      | 9.9  | 7.0  | 11.3 | 13.6 | 3.9  |
| Organic Acids               | 25.8                     | 18.5 | 9.2  | 12.7 | 12.6 | 18.2 |
| Amino Acids                 |                          |      |      |      |      |      |
| Neutral & Basic amino acids | 19.2                     | 39.0 | 44.7 | 41.8 | 34.6 | 37.0 |
| Acidic amino acids          | 34.1                     | 7.2  | 12.4 | 8.1  | 9.6  | 9.6  |
| Residue                     | 6.0                      | 12.0 | 11.3 | 10.3 | 10.5 | 10.5 |





TABLE 54

The Metabolism of acetate-2-C<sup>14</sup> by germinating Sunflower cotyledons,  
the neutral and basic amino acid fraction

Experimental procedure as in table 53a. Results expressed as percentage of total C<sup>14</sup>  
incorporated in neutral and basic amino acid fractions.

| Amino Acids<br>and amides | Age of Cotyledons (days) |      |      |      |      |
|---------------------------|--------------------------|------|------|------|------|
|                           | 1                        | 2    | 3    | 4    | 5    |
| Alanine                   | 100.0                    | 27.5 | 31.5 | 30.0 | 30.0 |
| Glycine                   | -                        | 1.2  | 2.3  | 4.8  | 1.9  |
| Methionine                | -                        | -    | 3.0  | 4.0  | 6.7  |
| Asparagine                | -                        | 3.0  | 2.6  | 2.7  | 2.3  |
| Glutamine                 | -                        | 60.0 | 54.2 | 45.0 | 49.5 |
| γ-amino butyric acid      | -                        | 8.3  | 6.2  | 13.5 | 9.6  |



TABLE 55

The Metabolism of acetate-2-C<sup>14</sup> by germinating Sunflower cotyledons, organic acid fraction

Experimental procedure as in table 53a.

| Organic Acids                             | Age of Cotyledons (days) |                     |           |                     |           |                     |
|-------------------------------------------|--------------------------|---------------------|-----------|---------------------|-----------|---------------------|
|                                           | 1                        |                     | 3         |                     | 5         |                     |
|                                           | C14 (cpm)                | % C14 incorporated* | C14 (cpm) | % C14 incorporated* | C14 (cpm) | % C14 incorporated* |
| Glycollate                                | 29,000                   | 6.4                 | 12,700    | 10.9                | 40,000    | 34.5                |
| Succinate                                 | 71,500                   | 15.7                | 12,400    | 10.7                | 16,000    | 13.8                |
| Malate                                    | 278,000                  | 61.2                | 42,400    | 36.6                | 37,200    | 32.0                |
| Citrate                                   | 55,500                   | 12.2                | 19,800    | 17.0                | 20,000    | 17.2                |
| Glyoxylate                                | -                        | -                   | 22,300    | 19.2                | -         | -                   |
| Others                                    | 20,000                   | 4.4                 | 6,400     | 5.5                 | 2,800     | 2.4                 |
| Total C14 incorporated into organic acids | 454,000                  |                     | 116,000   |                     | 116,000   |                     |

\* % C14 incorporated into organic acid fraction



biosynthesis of amino acids.

## 2. The Effect of Additions of Glyoxylate on Acetate-2-C<sup>14</sup> Metabolism in 5 Day Old Sunflower Cotyledons

If acetate is being metabolized by the reactions of the glyoxylate cycle in germinating sunflower cotyledons, additions of one of the component acids of the cycle might be expected to result in changes in the distribution of carbon-14 in the products of acetate-C<sup>14</sup> metabolism. For example, additions of glyoxylate might stimulate the incorporation of acetate carbon into malate and possibly also trap acetate carbon in the increased glyoxylate pool.

In order to investigate these possibilities, additions of unlabelled glyoxylate were made to tissue slices metabolizing acetate-2-C<sup>14</sup> (Table 56). Although additions of glyoxylate only slightly increased the total amounts of acetate-C<sup>14</sup> metabolism, the carbon-14 content of the organic acid fraction was increased. Furthermore, glyoxylate-C<sup>14</sup> was detected when glyoxylate additions were made. Table 57 shows that additions of glyoxylate also affected the distribution of carbon-14 in the neutral and basic amino acids as compared to the control. For example, glyoxylate additions led to a decrease in the amounts of carbon-14 entering glutamine, but gave a two-fold increase in the radioactivity present in glycine.

Additions of glyoxylate to tissues metabolizing acetate-C<sup>14</sup>, therefore, resulted in slight increases in the amounts of acetate metabolized, trapped carbon-14 in





TABLE 56

The Effect of glyoxylate on the metabolism of acetate-2-C14  
by cotyledons of germinating Sunflower seeds\*

0.5 g of cotyledon slices incubated at 30° for 6 hr. in darkness with 100 µmoles of phosphate buffer (pH 5.6) and 1 µmole of acetate containing 5 µc of C14.

| Fraction                    | Acetate-2-C14 |                       | Acetate-2-C14 +<br>5 µmoles glyoxylate |                       |
|-----------------------------|---------------|-----------------------|----------------------------------------|-----------------------|
|                             | C14 (cpm)     | % of incorporated C14 | C14 (cpm)                              | % of incorporated C14 |
| Carbon Dioxide              | 259,000       | 22.3                  | 319,000                                | 26.4                  |
| Lipids                      | 25,500        | 2.2                   | 33,400                                 | 2.7                   |
| Sugars                      | 105,000       | 9.0                   | 120,000                                | 9.9                   |
| Organic Acids               | 105,000       | 9.0                   | 142,000                                | 11.7                  |
| Amino Acids                 |               |                       |                                        |                       |
| Neutral & Basic amino acids | 425,500       | 36.7                  | 376,000                                | 31.0                  |
| Acidic amino acids          | 86,400        | 7.5                   | 62,000                                 | 5.1                   |
| Residue                     | 153,600       | 13.2                  | 160,600                                | 13.2                  |
| Total C14 incorporated      | 1,160,000     |                       | 1,213,000                              |                       |

\* 5 day old seedlings



TABLE 57

The Effect of glyoxylate on the metabolism of acetate-2-C<sup>14</sup> by germinating Sunflower cotyledons\*, neutral and basic amino acid fraction

Experimental procedure as in table 56.

| Amino Acids<br>and amides          | Acetate-2-C <sup>14</sup> |                                     | Acetate-2-C <sup>14</sup> + 5 $\mu$ moles glyoxylate |                                     |
|------------------------------------|---------------------------|-------------------------------------|------------------------------------------------------|-------------------------------------|
|                                    | C <sup>14</sup> (cpm)     | % of C <sup>14</sup> incorporated** | C <sup>14</sup> (cpm)                                | % of C <sup>14</sup> incorporated** |
| Asparagine                         | 9,500                     | 2.2                                 | 18,000                                               | 4.9                                 |
| Glycine                            | 12,500                    | 2.9                                 | 25,000                                               | 6.6                                 |
| Glutamine                          | 281,200                   | 66.1                                | 100,000                                              | 26.6                                |
| Alanine                            | 54,500                    | 12.8                                | 76,000                                               | 20.2                                |
| $\gamma$ -amino butyric acid       | 36,100                    | 8.5                                 | 62,000                                               | 16.4                                |
| Methionine                         | 18,700                    | 4.4                                 | 43,000                                               | 11.4                                |
| Others                             | 13,000                    | 3.0                                 | 52,000                                               | 13.9                                |
| Total C <sup>14</sup> incorporated | 425,500                   |                                     | 376,000                                              |                                     |

\* 5 day old cotyledons

\*\* C<sup>14</sup> incorporated into neutral and basic amino acid fraction



the glyoxylate pool added and increased the amounts of carbon-14 present in glycine. These findings are therefore consistent with the suggestion made earlier; that acetate carbon might be involved in amino acid biosynthesis via the partial reactions of the glyoxylate cycle.

#### Acetate-2-C<sup>14</sup> Metabolism in Germinating Watermelon Cotyledons

Three and five day old cotyledons of germinating watermelon seeds were incubated with acetate-2-C<sup>14</sup> for 6 hours (Table 58). The three day old cotyledons converted 26.5% of the total carbon-14 incorporated into the neutral and basic amino acid fraction. There was also considerable conversion of acetate-C<sup>14</sup> to carbon dioxide and the organic acids. Sugars, however, only accounted for 7.2% and 13.0% of the total carbon-14 incorporated by the 3 and 5 day old cotyledons respectively.

Analysis of the neutral and basic amino acid fraction (Table 59), showed that it consisted mainly of labelled  $\gamma$ -amino butyric acid, glutamine and alanine. Smaller amounts of activity were also recovered in asparagine and methionine.

#### Acetate-2-C<sup>14</sup> Metabolism in Germinating Pumpkin Cotyledons

As in the experiments with sunflower and watermelon cotyledons, acetate-2-C<sup>14</sup> was utilized by the pumpkin cotyledons, mainly for synthesis of amino acids (Table 60). There was also incorporation of carbon-14 into carbon dioxide, the insoluble residue and the organic acids. Considerable amounts of radioactivity were also present in the sugar fraction. Additions of 5  $\mu$ moles of non-radioactive glyoxylate enhanced the production of carbon dioxide and reduced the in-





TABLE 58

The Utilization of acetate-2-C14 by the cotyledons of germinating Watermelon seeds

0.5 g of cotyledon slices incubated at 30° for 6 hr. in darkness with 100 μmoles of phosphate buffer (pH 5.6) and 1 μmole of acetate containing 2 μc of C14.

| Fraction                    | 3 day old |                       | 5 day old |                       |
|-----------------------------|-----------|-----------------------|-----------|-----------------------|
|                             | C14 (cpm) | % of incorporated C14 | C14 (cpm) | % of incorporated C14 |
| Carbon Dioxide              | 36,000    | 21.7                  | 42,300    | 20.7                  |
| Lipids                      | 4,500     | 2.7                   | 4,000     | 2.0                   |
| Sugars                      | 12,000    | 7.2                   | 26,600    | 13.0                  |
| Organic Acids               | 29,500    | 17.7                  | 25,400    | 12.5                  |
| Amino Acids                 |           |                       |           |                       |
| Neutral & Basic amino acids | 44,000    | 26.5                  | 72,000    | 35.0                  |
| Acidic amino acids          | 14,400    | 8.7                   | 12,200    | 6.1                   |
| Residue                     | 25,600    | 15.5                  | 21,500    | 10.5                  |
| Total C14 incorporated      | 166,000   |                       | 204,000   |                       |



TABLE 59

The Metabolism of acetate-2-C<sup>14</sup> by germinating watermelon cotyledons;  
neutral and basic amino acid fraction

Experimental procedure as in table 58.

| Amino Acids<br>and amides | 3 day old cotyledons |                              | 5 day old cotyledons |                              |
|---------------------------|----------------------|------------------------------|----------------------|------------------------------|
|                           | <u>Cl4 (cpm)</u>     | <u>% of Cl4 incorporated</u> | <u>Cl4 (cpm)</u>     | <u>% of Cl4 incorporated</u> |
| Asparagine                | 1,400                | 3.2                          | 5,000                | 7.0                          |
| Glutamine                 | 7,200                | 16.3                         | 20,000               | 27.8                         |
| Alanine                   | 14,450               | 32.8                         | 7,660                | 10.6                         |
| γ-amino butyric acid      | 19,350               | 44.0                         | 39,340               | 54.7                         |
| Methionine                | 1,600                | 3.6                          | not active           | -                            |
| Total Cl4 incorporated    | 44,000               |                              | 72,000               |                              |



corporation of carbon-14 into the neutral and basic amino acid fraction. There was, however, little or no significant change in the amounts of carbon-14 entering the organic acids and sugars when glyoxylate was added.

Chromatographic analysis of the neutral and basic amino acid fraction (Table 61), revealed that it mainly consisted of labelled glutamine,  $\gamma$ -amino butyric acid and alanine. Apart from the overall decrease in the carbon-14 content of this fraction, additions of glyoxylate enhanced formation of  $\gamma$ -amino butyric acid, as was observed in the sunflower cotyledons.

In the organic acid fraction, succinate, malate, citrate and glycollate were all labelled following the six hour incubation period (Table 62). In the presence of glyoxylate, radioactivity was detected in glyoxylate.

Acetate metabolism in pumpkin cotyledons appears, therefore, to be similar to that shown to occur in the sunflower and watermelon tissues. In pumpkin cotyledons, the amino acids were the major compounds labelled from acetate- $C^{14}$  and the effect of glyoxylate additions, might indicate that acetate carbon is being metabolized by reactions involving production of glyoxylate.

#### Acetate-2- $C^{14}$ Metabolism in Germinating Linseed Cotyledons

In two day old linseed cotyledons (Table 63), there was a major incorporation of carbon-14 from acetate-2- $C^{14}$  into the neutral and basic amino acids. As in the other tissues examined for acetate- $C^{14}$  utilization, the organic acids and sugars were also labelled. Additions of 5  $\mu$ moles of non-radioactive glyoxylate enhanced the incorporation of carbon-14





TABLE 60

The Effect of glyoxylate on the metabolism of acetate-2-C14  
by cotyledons of germinating Pumpkin seeds\*

0.5 g of cotyledon slices incubated at 30° for 6 hr. in darkness with 100 μmoles of phosphate buffer (pH 5.6) and 1 μmole of acetate containing 2 μc of C14.

| Fraction                       | Acetate-2-C14 |                       | Acetate-2-C14 +<br>5 μmoles glyoxylate |                       |
|--------------------------------|---------------|-----------------------|----------------------------------------|-----------------------|
|                                | C14 (cpm)     | % of incorporated C14 | C14 (cpm)                              | % of incorporated C14 |
| Carbon Dioxide                 | 91,600        | 20.6                  | 116,600                                | 26.6                  |
| Lipids                         | 7,400         | 1.6                   | 17,000                                 | 3.9                   |
| Sugars                         | 31,900        | 7.2                   | 30,000                                 | 6.8                   |
| Organic Acids                  | 52,200        | 12.4                  | 58,400                                 | 13.3                  |
| Amino Acids                    |               |                       |                                        |                       |
| Neutral & Basic<br>amino acids | 149,500       | 33.7                  | 96,700                                 | 22.1                  |
| Acidic amino acids             | 29,900        | 6.7                   | 29,000                                 | 6.6                   |
| Residue                        | 80,500        | 18.1                  | 90,000                                 | 20.5                  |
| Total C14 incorporated         | 443,000       |                       | 437,700                                |                       |

\* 2 day old seedlings



TABLE 61

The Effect of glyoxylate on the metabolism of acetate-2-C<sup>14</sup> by germinating pumpkin cotyledons\*, neutral and basic amino acid fraction

Experimental procedure as in table 60.

| Amino Acids<br>and amides          | Acetate-2-C <sup>14</sup> |                                     | Acetate-2-C <sup>14</sup> + 5 $\mu$ moles glyoxylate |                                     |
|------------------------------------|---------------------------|-------------------------------------|------------------------------------------------------|-------------------------------------|
|                                    | C <sup>14</sup> (cpm)     | % of C <sup>14</sup> incorporated** | C <sup>14</sup> (cpm)                                | % of C <sup>14</sup> incorporated** |
| Asparagine                         | 1,900                     | 1.3                                 | 2,100                                                | 2.1                                 |
| Glycine                            | 1,500                     | 1.0                                 | 1,800                                                | 1.9                                 |
| Glutamine                          | 63,400                    | 42.4                                | 32,500                                               | 33.6                                |
| Alanine                            | 28,400                    | 18.9                                | 12,300                                               | 12.7                                |
| $\gamma$ -amino butyric acid       | 35,500                    | 23.8                                | 36,100                                               | 37.3                                |
| Leucine                            | 9,400                     | 6.3                                 | 7,800                                                | 8.1                                 |
| Others                             | 9,400                     | 6.3                                 | 4,100                                                | 4.2                                 |
| Total C <sup>14</sup> incorporated | 149,500                   |                                     | 96,700                                               |                                     |

\* 2 day old cotyledons

\*\* C<sup>14</sup> incorporated into neutral and basic amino acid fraction



TABLE 62

The Effect of glyoxylate on acetate-2-C<sup>14</sup> metabolism by germinating Pumpkin cotyledons\*, organic acid fraction

Experimental procedure as in table 60.

| Organic Acids                                          | Acetate-2-C <sup>14</sup> |                                                        | Acetate-2-C <sup>14</sup> +<br>5 $\mu$ moles glyoxylate |                                                        |
|--------------------------------------------------------|---------------------------|--------------------------------------------------------|---------------------------------------------------------|--------------------------------------------------------|
|                                                        | C <sup>14</sup> (cpm)     | % of C <sup>14</sup> of total<br>organic acid fraction | C <sup>14</sup> (cpm)                                   | % of C <sup>14</sup> of total<br>organic acid fraction |
| Glycollate                                             | 9,000                     | 17.2                                                   | 5,300                                                   | 9.1                                                    |
| Glyoxylate                                             | not active                | -                                                      | 12,300                                                  | 21.0                                                   |
| Succinate                                              | 14,000                    | 26.8                                                   | 11,300                                                  | 19.3                                                   |
| Malate                                                 | 9,400                     | 18.0                                                   | 11,000                                                  | 18.8                                                   |
| Citrate                                                | 11,000                    | 21.0                                                   | 9,500                                                   | 16.3                                                   |
| Glucose phosphate                                      | 2,300                     | 4.4                                                    | 2,000                                                   | 3.4                                                    |
| Unknown                                                | 3,900                     | 7.5                                                    | -                                                       | -                                                      |
| Others                                                 | 2,600                     | 5.0                                                    | 7,000                                                   | 12.0                                                   |
| Total C <sup>14</sup> incorporated<br>in organic acids | 52,200                    |                                                        | 58,400                                                  |                                                        |

\* 2 day old





from acetate-2-C<sup>14</sup> into the sugars by 5.2%. However, the other fractions isolated, were not apparently affected by this addition.

The neutral and basic amino acid fraction (Table 64), as in the watermelon and pumpkin cotyledons, contained labelled  $\gamma$ -amino butyric acid, glutamine and alanine. In addition, smaller amounts of radioactivity were recovered in asparagine and glycine.

The results of acetate feeding in this tissue are, therefore, similar to those obtained from experiments with the other fatty seeds investigated. In linseed, however, glyoxylate additions led to increases in the amounts of acetate carbon entering the sugar fraction.

The present studies of acetate metabolism have indicated that germinating fatty tissues actively utilize acetate for the synthesis of amino acids, amides, organic acids and also to a lesser extent for the biosynthesis of sugars. An appreciable amount of carbon-14, derived from acetate-2-C<sup>14</sup>, was recovered as carbon dioxide. It is clearly possible that if dark fixation of carbon dioxide occurs during the experimental period, some of the labelled products might be derived from this source. Furthermore, the distribution of carbon-14 in the amino acids, produced from acetate-C<sup>14</sup>, indicates certain basic differences from the amino acids produced from glyoxylate-C<sup>14</sup>. The organic acid fraction consisted essentially of the acids of the glyoxylate cycle, invariably with the greatest amounts of radioactivity being present in malate when acetate-2-C<sup>14</sup> was fed. There are, therefore, also differences



TABLE 63

The Effect of glyoxylate on the metabolism of acetate-2-C14 by germinating Linseed cotyledons\*

0.25 g of cotyledon slices incubated at 30° for 6 hr. in darkness with 100  $\mu$ moles of phosphate buffer (pH 5.6) and 1  $\mu$ mole of acetate-2-C14 containing 2  $\mu$ c of C14.

| Fraction                       | Acetate-2-C14 |                       | Acetate-2-C14 +<br>5 $\mu$ moles glyoxylate |                       |
|--------------------------------|---------------|-----------------------|---------------------------------------------|-----------------------|
|                                | C14 (cpm)     | % of incorporated C14 | C14 (cpm)                                   | % of incorporated C14 |
| Carbon Dioxide                 | 97,000        | 20.1                  | 103,500                                     | 21.1                  |
| Lipids                         | 11,000        | 2.2                   | 10,000                                      | 2.0                   |
| Sugars                         | 53,000        | 10.9                  | 79,000                                      | 16.1                  |
| Organic Acids                  | 50,800        | 10.5                  | 45,000                                      | 9.2                   |
| Amino Acids                    |               |                       |                                             |                       |
| Neutral & Basic<br>amino acids | 165,900       | 34.2                  | 168,000                                     | 34.2                  |
| Acidic amino acids             | 62,000        | 12.8                  | 30,800                                      | 6.3                   |
| Residue                        | 45,000        | 9.2                   | 54,000                                      | 11.0                  |
| Total C14 incorporated         | 484,700       |                       | 490,300                                     |                       |

\* 2 day old seedlings



TABLE 64

The Effect of glyoxylate on the metabolism of acetate-2-C<sup>14</sup> by germinating Linseed cotyledons\*, neutral and basic amino acid fraction

Experimental procedure as in table 63.

| Amino Acids<br>and amides          | Acetate-2-C <sup>14</sup> |                                     | Acetate-2-C <sup>14</sup> + 5 $\mu$ moles glyoxylate |                                     |
|------------------------------------|---------------------------|-------------------------------------|------------------------------------------------------|-------------------------------------|
|                                    | C <sup>14</sup> (cpm)     | % of C <sup>14</sup> incorporated** | C <sup>14</sup> (cpm)                                | % of C <sup>14</sup> incorporated** |
| Asparagine                         | 3,800                     | 2.3                                 | 6,500                                                | 3.8                                 |
| Glycine                            | 2,200                     | 1.3                                 | 2,600                                                | 1.5                                 |
| Glutamine                          | 51,800                    | 31.2                                | 41,600                                               | 24.8                                |
| Alanine                            | 47,300                    | 28.5                                | 41,500                                               | 24.7                                |
| $\gamma$ -amino butyric acid       | 46,700                    | 28.1                                | 65,900                                               | 39.2                                |
| Others                             | 14,100                    | 8.5                                 | 9,900                                                | 5.3                                 |
| Total C <sup>14</sup> incorporated | 165,900                   |                                     | 168,000                                              |                                     |

\* 2 day old cotyledons

\*\* C<sup>14</sup> incorporated into neutral and basic amino acid fraction





in the distribution of carbon-14 in the organic acids when acetate-C<sup>14</sup> is fed as compared to the earlier glyoxylate feeding experiments.

Despite these apparent differences, however, additions of glyoxylate did alter the distribution of carbon-14 in the various fractions isolated. Furthermore, the experiments with acetate-C<sup>14</sup> and non-radioactive glyoxylate indicated that trapping of acetate carbon, either as glyoxylate or an immediate metabolic product of glyoxylate, was occurring.



## Carbon Dioxide Fixation in Germinating Sunflower Cotyledons

In the experiments on glyoxylate and acetate metabolism in fatty seeds, carbon dioxide was always one of the major labelled fractions. There are several reports that such tissues have the ability to fix carbon dioxide in the dark (Benedict and Beevers 1961, Stiller, Neal and Beevers 1958, Bradbeer 1958). An attempt was, therefore, made to determine whether carbon dioxide fixation might play some role in the biosynthesis of amino acids in sunflower cotyledons.

Cotyledon slices, taken from a sample of germinating sunflower seeds, were incubated with carbon dioxide- $C^{14}$  at various stages of germination, as shown in Tables 65a and 65b. Fixation of carbon dioxide was greatest in the 5 day old cotyledons.

As germination proceeded, there was a noticeable change in the distribution of carbon-14 in the products of  $CO_2$  fixation. For example, in one day old tissues, the organic acids and acidic amino acids, together with the insoluble residue, contained the bulk of the carbon-14 fixed. As the cotyledons increased in age, the amount of carbon-14 entering the organic acids decreased while the radioactivity in the sugars and neutral and basic amino acids increased considerably (Table 65a, 65b).

As in the acetate feeding experiments, the neutral and basic amino acid fraction (Table 66) contained large amounts of labelled alanine, glutamine and  $\gamma$ -amino butyric acid. In addition, there was also some incorporation of carbon-14 into glycine and asparagine at all stages of germination.



TABLE 65a

The Fixation of  $\text{Cl}^{14}\text{O}_2$  by germinating Sunflower cotyledons

0.5 g slices of cotyledons incubated at  $30^\circ$  for 6 hr. in darkness with 100  $\mu\text{moles}$  of phosphate buffer (pH 5.6) and 1  $\mu\text{mole}$  of Sodium carbonate- $\text{Cl}^{14}$  containing 10  $\mu\text{c}$  of  $\text{Cl}^{14}$ . Radioactivity expressed as cpm.

| Fractions                           | Age of Cotyledons (days) |         |         |         |         |         |
|-------------------------------------|--------------------------|---------|---------|---------|---------|---------|
|                                     | 1                        | 2       | 3       | 4       | 5       | 6       |
| Lipids                              | 1,000                    | 700     | 1,300   | 4,000   | 4,000   | 3,000   |
| Sugars                              | 5,300                    | 5,200   | 10,500  | 35,200  | 62,000  | 52,000  |
| Organic Acids                       | 76,500                   | 43,000  | 38,000  | 55,000  | 88,000  | 71,000  |
| Amino Acids                         |                          |         |         |         |         |         |
| Neutral & Basic amino acids         | 8,000                    | 8,100   | 21,800  | 32,000  | 93,000  | 68,000  |
| Acidic amino acids                  | 36,400                   | 34,200  | 19,300  | 15,600  | 26,700  | 22,000  |
| Residue                             | 42,300                   | 31,800  | 35,300  | 48,200  | 76,000  | 61,000  |
| Total $\text{Cl}^{14}$ incorporated | 169,500                  | 123,000 | 126,200 | 190,000 | 350,000 | 277,000 |





TABLE 65b

The Fixation of  $\text{C}^{14}\text{O}_2$  by germinating Sunflower cotyledons

Experimental procedure as in table 65a. Results expressed as percentage of total  $\text{C}^{14}$  incorporated.

| Fractions                   | Age of Cotyledons (days) |      |      |      |      |      |
|-----------------------------|--------------------------|------|------|------|------|------|
|                             | 1                        | 2    | 3    | 4    | 5    | 6    |
| Lipids                      | 0.6                      | 0.5  | 1.0  | 2.1  | 1.1  | 1.1  |
| Sugars                      | 3.1                      | 4.2  | 8.3  | 18.5 | 17.9 | 18.8 |
| Organic Acids               | 45.1                     | 35.0 | 30.1 | 29.0 | 25.1 | 25.6 |
| Amino Acids                 |                          |      |      |      |      |      |
| Neutral & Basic amino acids | 4.7                      | 6.6  | 17.3 | 16.9 | 26.6 | 24.5 |
| Acidic amino acids          | 21.5                     | 27.9 | 15.3 | 8.2  | 7.6  | 8.0  |
| Residue                     | 25.0                     | 25.8 | 28.0 | 25.3 | 21.7 | 22.0 |



It is evident from Table 67, that malate was the most heavily labelled organic acid produced during  $C^{14}O_2$  fixation. However, the percentage of carbon-14 in malate decreased as germination proceeded and glycollate, succinate and citrate contained increasing percentages of the label. As in the acetate feeding experiments, the incorporation of carbon-14 into glycollate was extensive when the cotyledons were 5 days old.

The products of carbon dioxide fixation in these tissues are therefore similar to those produced from acetate. As in the earlier acetate feeding experiments, the label was extensively incorporated in the organic acids, notably into malate and glycollate. In addition, fixation of carbon dioxide into the amino acids occurred at all stages of germination.



TABLE 66

The Fixation of  $\text{Cl}^{14}\text{O}_2$  by germinating Sunflower cotyledons;  
neutral and basic amino acid fraction

Experimental procedure as in table 65a. Results expressed as percentage incorporation of  $\text{Cl}^{14}$  into the neutral and basic amino acid fraction.

| Amino Acids<br>and amides    | Age of Cotyledons (days) |      |      |      |      |      |
|------------------------------|--------------------------|------|------|------|------|------|
|                              | 1                        | 2    | 3    | 4    | 5    | 6    |
| Glycine                      | 11.8                     | 6.5  | 4.6  | 4.1  | 4.7  | 5.2  |
| Alanine                      | 14.7                     | 34.0 | 24.1 | 25.4 | 29.0 | 18.3 |
| Glutamine                    | 49.7                     | 41.5 | 62.3 | 60.5 | 55.3 | 67.9 |
| Asparagine                   | 13.5                     | 15.5 | 6.0  | 6.8  | 6.7  | 5.2  |
| $\gamma$ -amino butyric acid | 10.3                     | 2.4  | 3.0  | 3.1  | 4.1  | 3.4  |





TABLE 67

The Fixation of  $C^{14}O_2$  by germinating Sunflower cotyledons,  
organic acid fraction

Experimental procedure as in table 53a. Results expressed  
as percentage of  $C^{14}$  incorporated into organic acid fraction.

| <u>Organic Acids</u> | <u>Age of Cotyledons (days)</u> |          |          |
|----------------------|---------------------------------|----------|----------|
|                      | <u>1</u>                        | <u>3</u> | <u>5</u> |
| Malate               | 96.4                            | 42.0     | 56.6     |
| Succinate            | 2.3                             | 20.0     | 6.3      |
| Citrate              | 1.2                             | 13.3     | 8.6      |
| Glycollate           | -                               | 24.6     | 25.8     |
| Glucose phosphate    | -                               | -        | 2.6      |



## DISCUSSION

Whilst the significance of certain observations has been discussed in the appropriate sections, it is proposed in this section to consider their implications in more detail under the following sub-headings.

### Formate Production

In the present studies, evidence was obtained that glyoxylate and glycine can be converted to formate by sunflower cotyledons and carrot storage tissues. Although labelled formate was produced from both substrates, glyoxylate appeared to be a better precursor (Table 2). If the mechanism for the production of formate is similar to that shown to occur in animal tissues, it might be presumed that this compound arises exclusively from the 2 position of glyoxylate as a result of oxidative decarboxylation. Glycine would, therefore, only give rise to formate after conversion to glyoxylate. Consequently, glyoxylate might be a better precursor of formate in these tissues.

### Formate Utilization

#### 1. Conversion to Carbon Dioxide and Formic Dehydrogenase Activity

The results presented in Tables 3-14 clearly indicate that in all the tissues examined, carbon dioxide was the chief product of formate- $C^{14}$  metabolism. This might indicate a major role of formic dehydrogenase in the utilization of formate by the tissues examined. This possibility is supported by several reports (Davies 1956, Davison 1949, Mazelis 1960) of the widespread distribution of this en-



zyme in plant tissues. Also, in the present investigations, formic dehydrogenase was present in extracts prepared from germinating pea cotyledons, castor bean endosperm and corn roots (Tables 19-22). In both kinds of germinating seeds examined, formic dehydrogenase activity fluctuated during the germination period. Studies with castor bean endosperm indicated that the oxidation of formate was stimulated by additions of NAD and NADP. Furthermore, studies on the intracellular localization of this enzyme indicate that it is mainly concentrated in the cytoplasm of castor bean endosperm.

Several workers (see Review of Literature) have suggested that formate is utilized by plants following its oxidation to carbon dioxide. Formate metabolism might, therefore, involve the processes of photosynthesis and dark CO<sub>2</sub> fixation. It is evident from Tables 3 and 4 that in addition to extensive oxidation of formate to carbon dioxide, there was a considerable formation of labelled amino acids in castor bean endosperm. These tissues are known to fix carbon dioxide in the dark; the main products of this fixation being organic acids and sugars (Benedict and Beevers 1961, Stiller, Neal and Beevers 1958).

These observations might suggest that formate utilization in this tissue occurs by reactions other than carbon dioxide fixation. However, the possibility that some formate carbon is incorporated into the tissues as carbon dioxide cannot be entirely eliminated.





In addition to this, it might also be argued that if the utilization of formate- $C^{14}$  mainly involves assimilation of carbon dioxide, this intermediate might conceivably be incorporated into photosynthetic products by tissues containing chlorophyll. Such photosynthetic assimilation of formate carbon might result in greater amounts of formate being utilized in the light than would occur in darkness.

In the present investigations, formate metabolism was not stimulated when the tissues were illuminated. For example, in pea leaves (Table 11), formate- $C^{14}$  was mainly oxidized to carbon dioxide in the light and dark. However, the percentage of carbon-14 evolved as carbon dioxide was decreased when the tissues were illuminated. Also, illumination of the tissues altered the distribution of carbon-14 in the other fractions isolated. Increases in the radioactivity present in sugars and neutral and basic amino acids were obvious. These changes in the pattern of distribution of formate carbon might, therefore, indicate that formate utilization in the light may involve some photosynthetic assimilation of  $C^{14}O_2$  produced by formate oxidation.

When the distribution of carbon-14 from formate- $C^{14}$  and carbonate- $C^{14}$  was compared (Table 12), it was clear that the utilization of these compounds in the dark may have involved the participation of different pathways. The utilization of both compounds by corn coleoptiles resulted in labelling of the amino acid and organic acid fractions. However, the percentage of carbon-14 entering



the amino acids was considerably higher when formate was the substrate. The percentage of carbon-14 entering the organic acid fraction was greater from carbonate-C<sup>14</sup> than from formate-C<sup>14</sup>. Although these observations may be interpreted as indicating that formate and carbonate are utilized by essentially different metabolic pathways, additions of unlabelled carbonate during formate-C<sup>14</sup> feeding (Table 12), did result in a very marked reduction in the total carbon-14 incorporated. This would apparently indicate that some of the formate was utilized following oxidation to carbon dioxide.

## 2. The Role of Formate in Amino Acid Biosynthesis

In addition to extensive oxidation to carbon dioxide, formate was readily incorporated into amino acids such as serine, methionine and methionine sulphoxide. Degradations of the serine produced from formate-C<sup>14</sup> feeding showed that heavy labelling of the 3 position had occurred. It is possible, therefore, that formate is involved in a transmethylation reaction similar to that demonstrated in animal tissues (Alexander and Greenberg 1955).

These conclusions are further supported by the studies on the effects of homocysteine and glycine on formate metabolism. It has been demonstrated in Tables 15-18, that additions of glycine enhanced the incorporation of formate into serine. Similarly, homocysteine increased the production of methionine and methionine sulphoxide. However, in these experiments, formate carbon was also incorporated into homocysteine. Degradations of this homo-





cysteine showed that its carboxyl group contained only 3% of the total radioactivity present in the molecule. Studies with radiosulphate, using these tissues (Sinha and Cossins 1963), have shown that sulphur-35 is readily incorporated into cysteine and cysteic acid. With the increase in the experimental period, the label appeared in homocysteine and methionine. It is possible therefore that these inter-conversions involved a transthiolation reaction in which homocysteine was an important intermediate (see fig. 2). If these reactions are occurring as suggested by the figure, the carbon skeleton of homocysteine would be derived from homoserine.

In the present experiments, labelling of homocysteine from formate-C<sup>14</sup> might be explained if homoserine was labelled. Large amounts of homoserine-C<sup>14</sup> are produced during CO<sub>2</sub> fixation in pea cotyledons (Cossins 1964), presumably arising via aspartate (Larson and Beevers 1963). Homocysteine might therefore become labelled from carbon dioxide as suggested in figure 2. Carbon dioxide produced from formate oxidation would be incorporated into the 4 position of oxaloacetate, aspartate and homocysteine (Webster 1959). Clearly, if this pathway operates for the formation of homocysteine-C<sup>14</sup> from formate-C<sup>14</sup>, labelling of the 1 position would be very slight, unless extensive randomization of the label in oxaloacetate occurred via fumarase activity. However, only further detailed studies would fully justify the validity of this assumption.

The results of formate-C<sup>14</sup> experiments do, however,





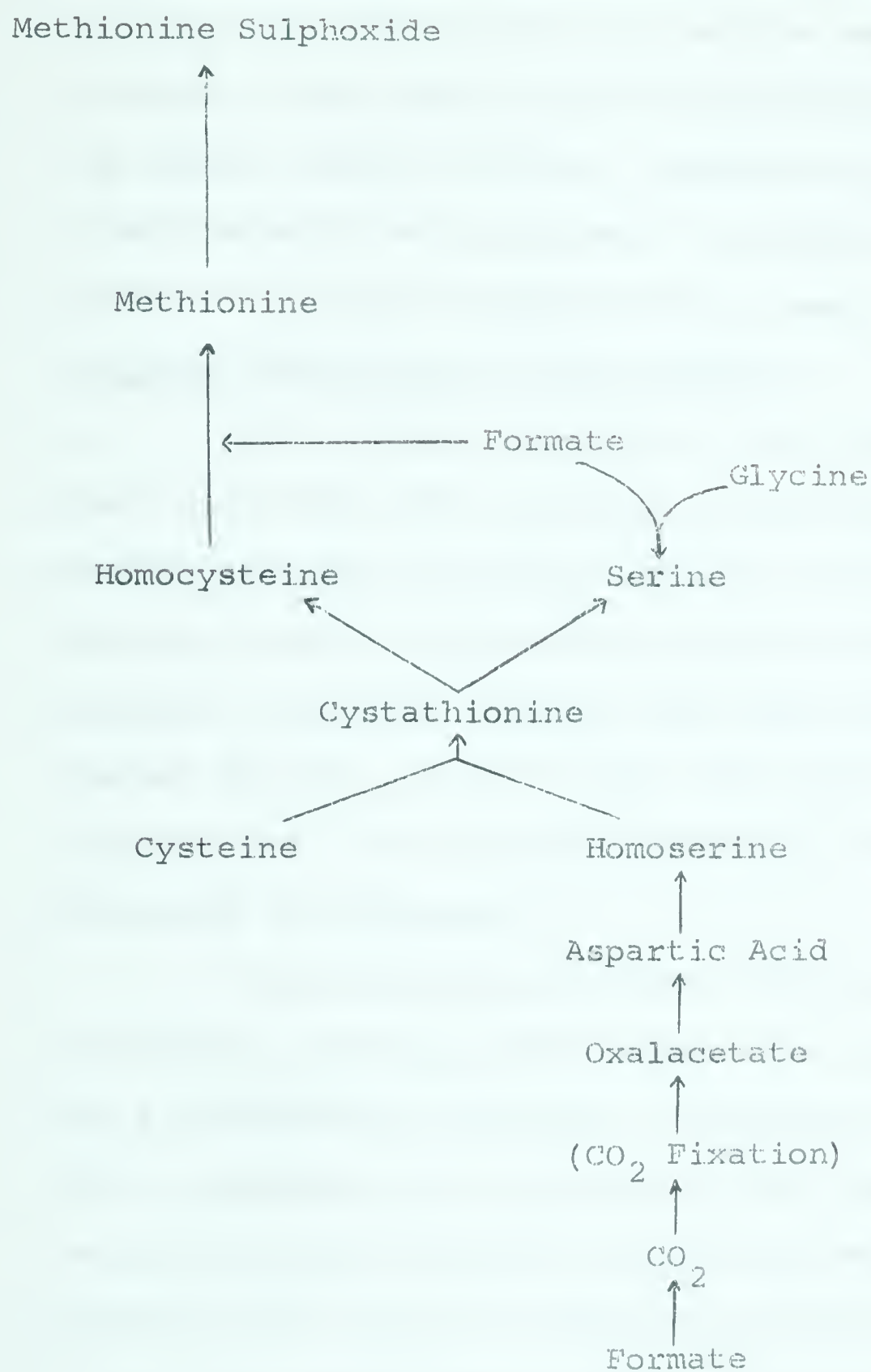


Figure 2

Possible Pathways for the Utilization of Formate in Serine and Methionine Biosynthesis



indicate that this compound plays an important role in pathways for the synthesis of serine and methionine. Studies of the intramolecular distribution of carbon-14 in serine and the 'sparker' effects of glycine and homocysteine might be interpreted as indicating that these pathways involve transmethylation reactions.

#### Glycine Metabolism by Plant Tissues

The results presented in the present investigations have indicated that the plant tissues examined rapidly metabolized the micromolar amounts of glycine-C<sup>14</sup> supplied. In all tissues, serine was one of the chief products of glycine metabolism together with the production of labelled carbon dioxide. In all cases, the organic acids became radioactive, with the greatest amounts of carbon-14 being detected in glyoxylate.

The formation of serine from glycine requires a carbon-one compound which yields the 3 position of serine by a condensation involving tetrahydrofolate (Sakami 1955). Such a reaction is reversible and has been demonstrated to occur in animal tissues (Kisliuk and Sakami 1955) and in certain plant tissues (Wilkinson and Davies 1958).

In the present investigations, it has been demonstrated that formate can give rise to the 3 position of serine. These results are in agreement with those of Tolbert (1955) and McConnell and Bilinski (1959).

In the present experiments, the 3 position of serine was readily formed from glycine by carrot tissues (Table 28). Over 50% of the carbon-14 present in the



isolated serine was in the 3 position. It appears possible, therefore, that glycine following conversion to glyoxylate might be split into one carbon fragment which can become the 3 position of serine. The 1 and 2 positions of serine would be derived from the corresponding positions of the glycine molecule. A glyoxylate transaminase system has been shown to exist in carrot tissue (page 99). Furthermore, glycine is readily converted into formate by these tissues (page 28). However, data obtained from the present feeding experiments might indicate that the 3 position of serine might arise more directly from glycine rather than via glyoxylate formation. For example, a greater percentage of  $C^{14}$  entered the 3 position of serine in the glycine- $C^{14}$  feeding experiments than was found when glyoxylate- $C^{14}$  was supplied. A possible reason for this might lie in the oxidation level of the one carbon fragment which will become the 3 position of serine. Clearly, if the  $\alpha$ -carbon of glycine is more readily converted into the carbon-one radical than the 2 position of glyoxylate, glycine would be a better precursor of the 3 position of serine.

Recently, Wang and Burris (1963) have also discussed the possibility that a direct oxidation of glycine to carbon dioxide and a carbon-one unit might occur in higher plants. This direct cleavage of the glycine molecule has been demonstrated to occur in Diplococcus glycinophilus (Sagers and Gunsalus 1961). If such a pathway were operating, the carbon-one unit would presumably be more reduced than formate and therefore might be a better precursor for the 3 position of serine.





Therefore, the present investigations have shown that glycine-1,2-C<sup>14</sup> can give rise to all carbons of serine. However, it is difficult to assess whether the 3 position of serine arises exclusively via oxidation of glyoxylate produced from glycine, or in part from a direct oxidation of glycine as occurs in *Diplococcus glycinophilus*.

In addition to serine biosynthesis, glycine metabolism, in all the tissues examined, resulted in labelling of the organic acids (Tables 23, 25). In all cases, the greatest amounts of carbon-14 were present in glyoxylate. As was shown in the section on glyoxylate transaminase (page 108), this glyoxylate could arise from glycine in a transamination reaction.

The castor bean endosperm slices incorporated small amounts of carbon-14 into glycollate, malate and succinate. Labelling of malate and succinate could readily occur from glyoxylate-C<sup>14</sup> by a series of reactions involving malate synthetase, fumarase and isocitratase (Beevers 1961a). However, due to the very low percentage of carbon-14 incorporated into the organic acids, other than glyoxylate, it appears that the utilization of glyoxylate derived from glycine does not involve organic acid metabolism to any great extent. Furthermore, this glyoxylate was not extensively converted to carbohydrate.

Labelling of malate, succinate, citrate and the pyruvate fraction was observed in the carrot tissues following periods of glycine metabolism (Table 25). Although there is no definite information regarding the occurrence of malate



synthetase in carrot tissues, labelling of these acids may have resulted from subsequent glyoxylate metabolism. Alternatively, labelling of the organic acids could occur via  $\text{CO}_2$  fixation. Such carboxylation reactions leading to malate formation are of fairly widespread occurrence in higher plant tissues (Walker 1962). Finally, pyruvate or hydroxypyruvate might arise from serine by a transamination reaction (Willis and Sallach 1963). These pathways would conceivably yield products containing carbon-14 derived originally from glycine. By operation of the TCA cycle, the label might be further distributed among the organic acids isolated.

In carrot and castor bean endosperm, the intramolecular distribution of carbon-14 in the glycine molecule was greatly altered during the experimental period (Table 28). Labelled glycine, extracted from the tissues after incubation for 6 hours at  $30^\circ$ , contained a higher percentage of carbon-14 in the 2 position than was observed at the start of the experiment. It appears, therefore, that considerable breakdown and resynthesis of glycine had occurred during the experiments.

Resynthesis of glycine via ethanolamine might possibly account for the increased carbon-14 content of the 2 position of the glycine extracted from the carrot tissues. There is evidence from studies with animal tissues that glycine can be converted to ethanolamine in vivo, involving the intermediary formation of serine which is decarboxylated (Elwyn, Weissbach, Henry and Sprinson 1955). The ethanolamine so formed can then be converted to glycine via glycoaldehyde, glycollate and glyoxylate (Fruton and Simmonds 1958). Operation of this





'glycine-ethanolamine cycle' (fig. 3) thus leads to the conversion of the 3 position of serine to the 2 position of glycine. Thus in carrot tissues, where the 3 position of serine contained 54% of the carbon-14 present in the molecule (Table 28), operation of this cycle might lead to production of glycine with a greater percentage of carbon-14 in the 2 position.

In the castor bean endosperm, however, the enrichment of the 2 position of glycine during the experiments cannot be explained on the basis of a glycine-ethanolamine cycle. In these tissues, the 3 position of serine only contained 7% of the carbon-14 content of the molecule (Table 28). If this serine were involved in the reactions shown in figure 3, glycine more heavily labelled in the carboxyl carbon would be produced. Thus the changes in the intramolecular distribution of carbon-14 in glycine extracted from the castor bean tissues cannot be readily explained on the basis of established pathways for glycine metabolism and synthesis.

#### Glyoxylate Metabolism by Plant Tissues

The present studies have shown that in all the tissues examined, glyoxylate is involved in the biosynthesis of glycine and serine. In addition, a considerable amount of carbon-14 from glyoxylate-C<sup>14</sup> was recovered in carbon dioxide, the organic acids and the insoluble residue which mainly consisted of amino acids (Table 68).

The biosynthesis of glycine from glyoxylate would require an addition of an amino group from a suitable amino donor, the reaction being one of transamination. A glyoxylate





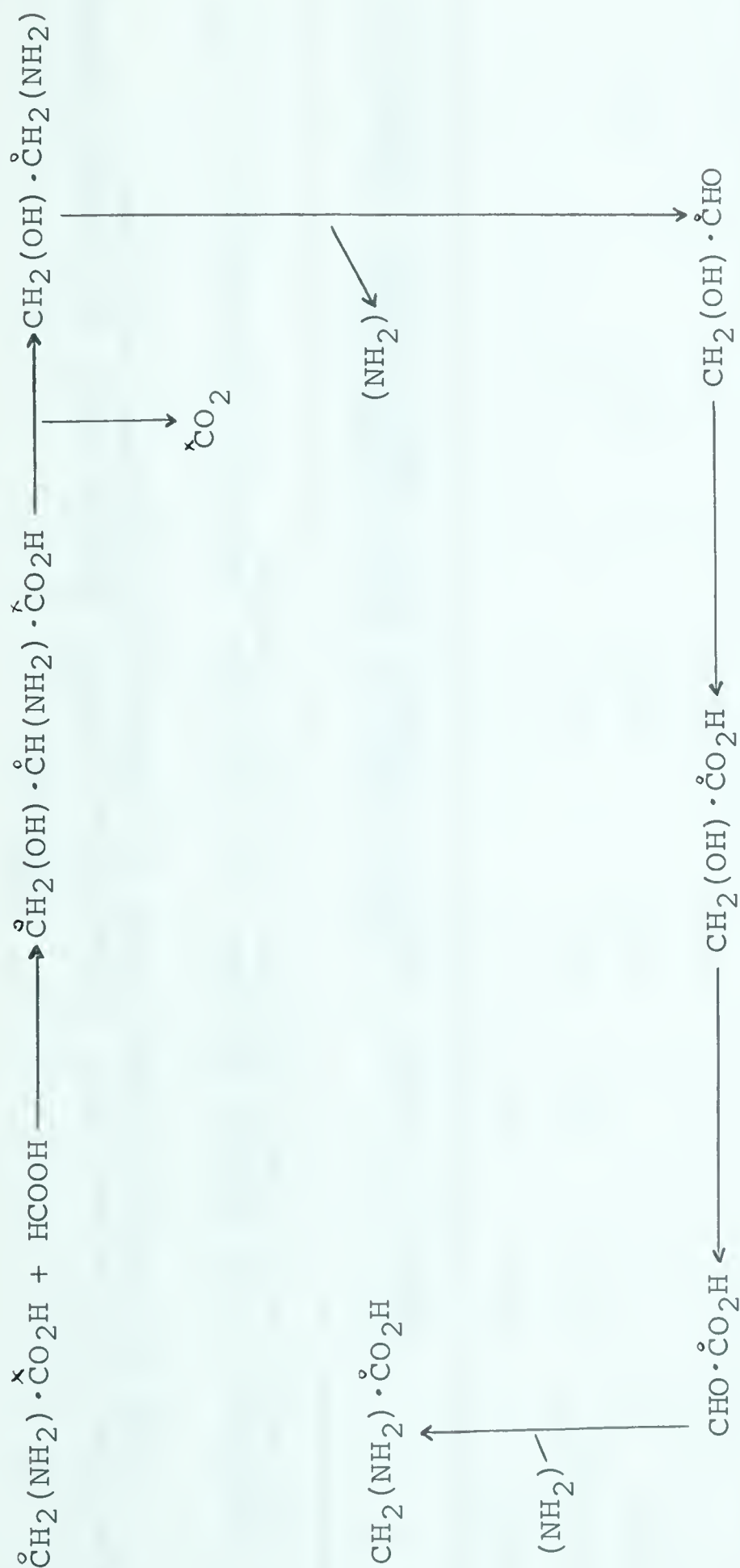


Figure 3

Ethanolamine Cycle for Rearrangement of Carbon Atoms in Glycine



TABLE 68

The Incorporation of glycine-2-C14, glyoxylate-1,2-C14, acetate-2-C14 and carbon dioxide-C14 into the protein amino acids of germinating Sunflower cotyledons

Experimental procedure as in tables 29a, 38a, 53a, and 65a. Results expressed as % of C14 incorporated into amino acid fraction of the insoluble residue.

| Substrate            | Amino Acids in Protein Hydrolysate |               |                |                      |                      |                   |               |
|----------------------|------------------------------------|---------------|----------------|----------------------|----------------------|-------------------|---------------|
|                      | <u>Glycine</u>                     | <u>Serine</u> | <u>Alanine</u> | <u>Glutamic Acid</u> | <u>Aspartic Acid</u> | <u>Methionine</u> | <u>Others</u> |
| *Glycine-2-C14       | 58.4                               | 41.6          | -              | -                    | -                    | -                 | -             |
| **Glyoxylate-1,2-C14 | 30.6                               | 18.9          | 11.1           | 18.5                 | 7.9                  | -                 | 12.9          |
| *Acetate-2-C14       | -                                  | -             | 21.9           | 34.4                 | 17.1                 | 13.4              | 13.1          |
| *C14O2               | -                                  | -             | 20.5           | 36.1                 | 25.8                 | -                 | 17.6          |

\* 5 day old cotyledons

\*\* 4 day old cotyledons



transaminase system has been shown to occur in a variety of plant tissues including carrot storage tissues, corn coleoptiles, pea leaves and sunflower cotyledons. Furthermore, the experiments indicated that several amino acids could be utilized in this transamination reaction. Because of the widespread occurrence of the enzyme and its relatively high activity, it is possible that this reaction may be of considerable importance in glycine biosynthesis in vivo.

As mentioned earlier, the presence of glyoxylate transaminase systems has been demonstrated in microorganisms (Campbell 1956) and in *Phaseolus radiatus* (Sastry and Ramakrishnan 1961). The conversion of glyoxylate to glycine in vivo has also been shown to occur in silkworms (Muramatsu and Shimura 1962) and in tomato fruits (Doyle, Huff and Wang 1960).

The incorporation of glyoxylate into all the carbon atoms of serine would presumably require at least two enzyme catalyzed steps. Firstly, glyoxylate could be converted to glycine by a transamination reaction. Secondly, a carbon-one unit, possibly arising from glyoxylate, might condense with glycine to give rise to the 3 position of serine in a transmethylation reaction. The origin and chemical structure of the carbon-one unit is still somewhat controversial (see page 150). However, in the present studies, production of formate from glyoxylate has been successfully demonstrated. Furthermore, small amounts of carbon-14 from glyoxylate entered the 3 position of serine (Table 36) in the various tissues examined. Therefore, it is possible that glyoxylate may be





decarboxylated giving rise to formate. The latter compound might then become the 3 position of serine by a series of reactions involving the intermediary formation of formyltetrahydrofolate.

The organic acid fraction, following glyoxylate- $C^{14}$  feeding was invariably found to contain glycollate and oxalate. In time course studies carried out with carrot tissues, glycollate was heavily labelled after 2 minutes (Table 31). The presence of a glyoxylic acid reductase system has been demonstrated in some plant tissues (Zelitch 1955). It is possible that this enzyme system was responsible in the present experiments for the considerable reduction of glyoxylate to glycollate that was invariably observed in these tissues. Oxalate- $C^{14}$ , produced during the experimental period, might be a direct oxidation product of glyoxylate- $C^{14}$  as has been demonstrated by Millerd, Morton and Wells (1963) to occur in Oxalis pes-caprae. Other organic acids such as malate, succinate and citrate may arise from glyoxylate by a pathway involving malate synthetase or where this enzyme is absent by  $CO_2$  fixation. Dark fixation of carbon dioxide into organic acids, particularly malate, is now well known (Walker 1962).

From the present work, it can be concluded that glyoxylate can be metabolized in the plant tissues investigated by several different pathways (fig. 4). The relative importance of these various pathways undoubtedly varies with the tissue examined.

In all cases, however, the tissues used in the feeding



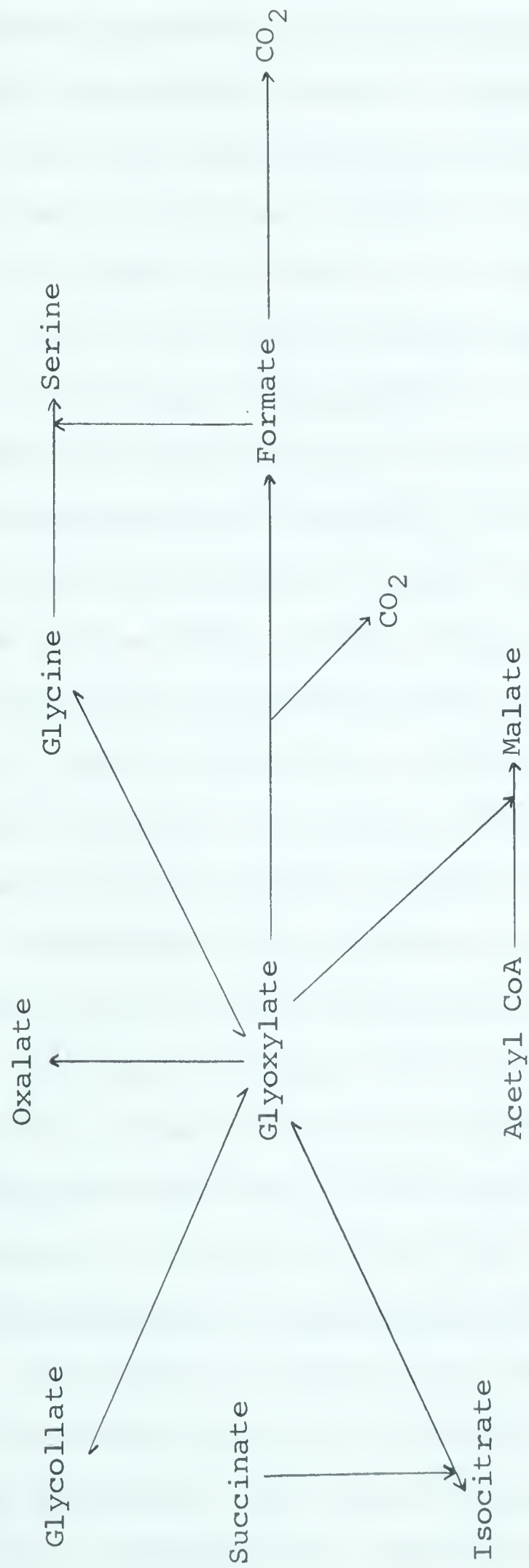


Figure 4

Possible Pathways for the Metabolism of Glyoxylate in Plant Tissues



experiments mainly utilized the supplied glyoxylate for the biosynthesis of amino acids and proteins. In addition, this compound was readily reduced to glycollate, oxidized to oxalate and also decarboxylated to carbon dioxide and formate. Formate may be further oxidized to carbon dioxide or might be used as a carbon-one unit for the synthesis of serine.

In all the fatty tissues examined, there was, however, a very minor incorporation of glyoxylate into the sugar fraction, even though there is presumably also conversion of fat to carbohydrates occurring in these tissues. The apparent minor role of glyoxylate in sugar biosynthesis might be explained on the basis of the intracellular localization of the glyoxylate cycle enzymes and the glyoxylate transaminase system. Malate synthetase is presumably located in the mitochondria (Yamamoto and Beevers 1960). The glyoxylate transaminase system is, however, mainly concentrated in the cytoplasm. Therefore, it is possible that a part of the exogenously supplied glyoxylate may be converted to glycine in the cytoplasm, leaving only small amounts for condensation with acetyl CoA. However, further studies are necessary to assess the relative importance of the various pathways by which glyoxylate may be utilized by these tissues.

#### Acetate Metabolism in Germinating Seeds

The present studies have shown that amino acids are one of the major products of acetate-2-C<sup>14</sup> metabolism in the tissues examined. This fraction invariably contained labelled glutamine, glutamic acid, alanine,  $\gamma$ -amino butyric acid, aspartic acid and asparagine. Some of these amino acids, including





aspartic acid, glutamic acid and alanine, are also the chief labelled components of the insoluble residue (Table 68). Therefore, it can be concluded that acetate is mainly utilized for the biosynthesis of amino acids and proteins in these tissues.

It has been suggested by various workers that acetate-2-C<sup>14</sup> is extensively utilized in the biosynthesis of sugars during the germination of fat storing seeds. There is now considerable evidence that the glyoxylate cycle is involved in sugar biosynthesis (Beevers 1961). As mentioned earlier, growing tissues might also use acetate for the synthesis of amino acids. In fact, the glyoxylate cycle could be an excellent pathway for amino acid biosynthesis. During the operation of the glyoxylate cycle, one of the most important features is the conservation of carbon. This is in marked contrast to the operation of the TCA cycle where the reactions are essentially catabolic and carbon is lost as carbon dioxide. If the glyoxylate cycle operates as suggested (Beevers 1961), the intermediary formation of glyoxylate could lead to the synthesis of malate without any loss of acetate carbon as carbon dioxide. If this malate is now metabolized via the partial reactions of the TCA cycle, carbon could now be drained off at  $\alpha$ -ketoglutarate and utilized in the synthesis of glutamic acid, glutamine or  $\gamma$ -amino butyric acid. This pathway, if operating, would conserve 5 carbon atoms in glutamic acid with the release of only one molecule of carbon dioxide. In the present experiments, glutamine and  $\gamma$ -amino butyric acid were found to be the chief products of acetate metabolism in



watermelon, linseed, sunflower and pumpkin cotyledons. Furthermore, additions of non-radioactive glyoxylate to the tissues incubated with acetate- $C^{14}$  enhanced the incorporation of carbon-14 into the organic acid fraction in most tissues and also acetate carbon was trapped in the glyoxylate pool. These observations are consistent with the glyoxylate cycle operation where acetate carbon, incorporated into malate in the malate synthetase reaction, is released as glyoxylate in the isocitratase reaction. It might, therefore, be concluded that the glyoxylate cycle is operating in these tissues and plays a major role in the synthesis of amino acids.

These conclusions can be summarized as shown in figure 5. The essential feature of acetate metabolism in these tissues appears to be its involvement in the synthetic reactions whereby there is considerable conservation of carbon.

#### The Role of Carbon Dioxide Fixation in Amino Acid Biosynthesis

It has been suggested by various workers that the TCA cycle provides a mechanism for the biosynthesis of certain amino acids such as glutamate and aspartate. These amino acids in turn may be used in the synthesis of other amino acids and amides such as histidine, proline, hydroxyproline, threonine, homoserine, glutamine, asparagine and  $\gamma$ -amino butyric acid (Webster 1959). A net synthesis of these amino acids would, however, require a net gain of carbon. It is well established that the organic acids of the TCA cycle are in a dynamic equilibrium. A drain of any component acid such as oxaloacetate or  $\alpha$ -ketoglutarate would, therefore, disturb this equilibrium unless the cycle acids can be supplemented with carbon exactly



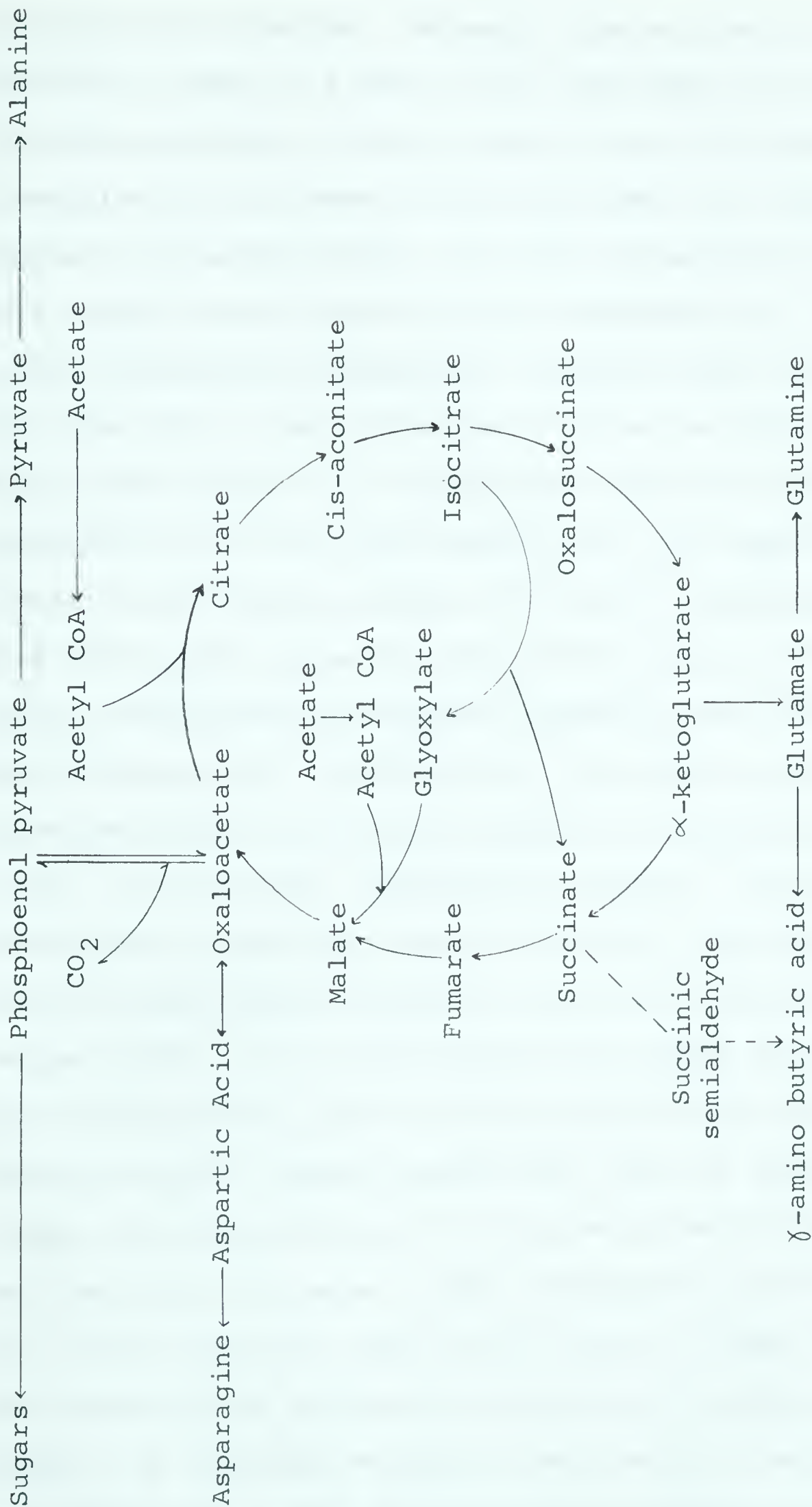


Figure 5

Possible Pathways for Acetate Metabolism in Germinating Fatty Seeds







balancing that removed. Normally, during the TCA cycle operation, there is a loss of two molecules of carbon dioxide for every molecule of acetyl CoA entering the cycle. If  $\alpha$ -ketoglutarate is removed from the cycle and utilized in the synthesis of amino acids, this will correspond to a removal of 5 carbon atoms from the cycle intermediates. Similarly, in the synthesis of aspartate, 4 carbon atoms will be lost from the cycle. The synthesis of these two amino acids, therefore, could result in a considerable drain of carbon. Clearly, operation of the TCA cycle alone could not support a net synthesis of these amino acids. It has been emphasized earlier that in tissues, known to contain the enzymes of the glyoxylate cycle, conservation of carbon is possible and this could be used to supplement the TCA cycle. In addition to this mechanism, dark  $\text{CO}_2$  fixation might also supplement the cycle acids. In this case, phosphoenol pyruvate, required in the carboxylation reaction, could be derived from glycolysis. Since the reactions of dark  $\text{CO}_2$  fixation are well established (Walker 1962), it is conceivable that carbon dioxide might play a significant role in amino acid biosynthesis. In the present studies, carbon dioxide was fixed by germinating sunflower cotyledons during the 6 day experimental period. There was a striking increase in the incorporation of carbon-14 into the neutral and basic amino acid fraction (Table 65b), consisting mainly of glutamine and alanine, as germination proceeded. In the early stages of germination, the organic acids and acidic amino acids were the chief products of  $\text{CO}_2$  fixation. Incorporation of carbon-14 into the sugar fraction also in-



creased to a maximum in the 5 day old cotyledons. These results, therefore, indicate that the tissues can utilize carbon dioxide during early stages of germination and can incorporate this carbon into a variety of cellular components.

Since the organic acid fraction consisted mainly of malate (Table 67), it is possible that carbon was fixed by a series of reactions similar to those reported to occur in Crassulacean tissues (Ranson and Thomas 1960). If this carboxylation reaction is extensive and has a high affinity for carbonate, as is suggested by the  $C^{14}O_2$  feeding experiments, it is possible that considerable amounts of malate could be synthesized by this mechanism. This malate might now be converted to alanine if randomization of the label occurs via fumarase activity. Similarly, malate carbon might be converted into aspartate, glutamate, glutamic and  $\gamma$ -amino butyrate by the partial reactions of the TCA cycle.

The present studies have indicated that considerable biosynthesis of amino acids occurs in the tissues examined. Feeding experiments using certain carbon-one and carbon-two compounds have indicated the importance of these substrates in these biosynthetic reactions.





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